# SEROPREVALENCE OF BRUCELLOSIS AMONG ANIMAL HANDLERS AND ANALYSIS OF RISK FACTORS

**DISSERTATION SUBMITTED FOR**

**M.D. (BRANCH IV)**

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This is to certify that the Dissertation "**SEROPREVALENCE OF BRUCELLOSIS AMONG ANIMAL HANDLERS AND ANALYSIS OF RISK FACTORS"** presented herein by **Dr. S.NIRMALADEVI** is an original work done in the Department of Microbiology, Tirunelveli Medical College Hospital, Tirunelveli for the award of Degree of M.D. (Branch IV) Microbiology under my guidance and supervision during the academic period of 2010 - 2013.

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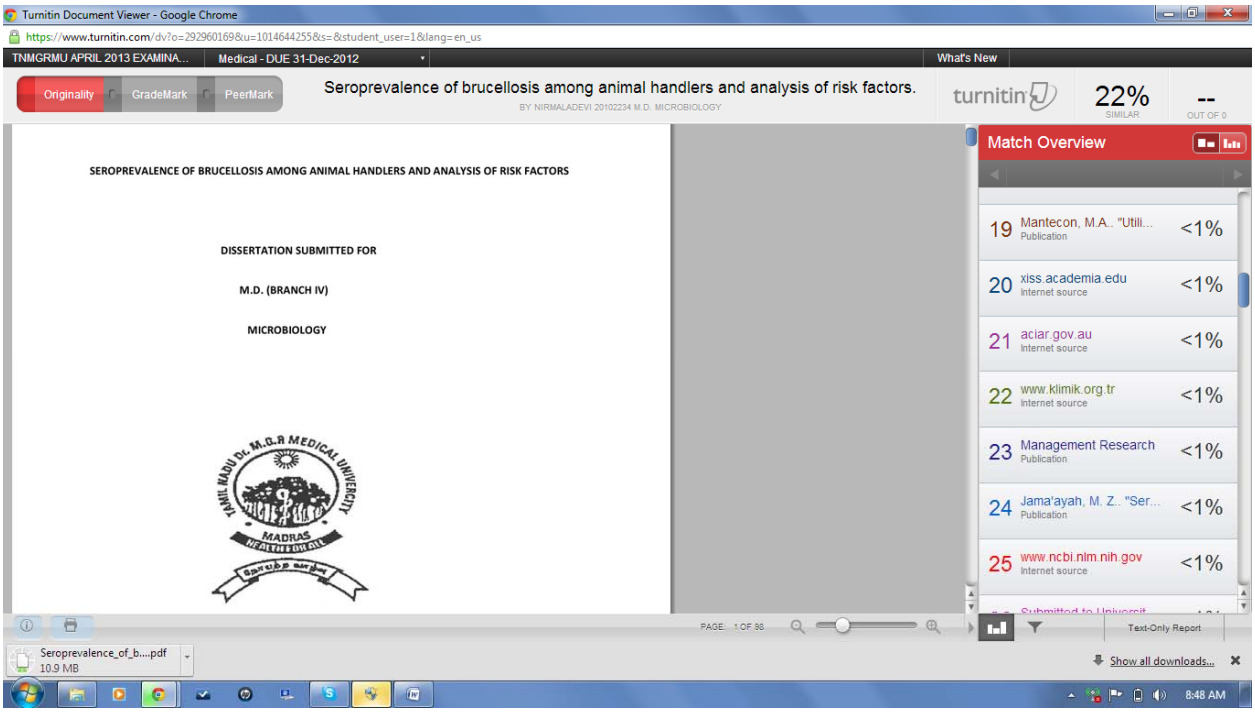
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The dissertation is submitted to The Tamilnadu Dr. M.G.R.Medical University towards the partial fulfilment of requirements for the award of M.D. Degree (Branch IV) in Microbiology.

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# ABBREVIATIONS

| | | |
|---|---|---|
| WHO | - | WORLD HEALTH ORGANIZATION |
| STAT | - | STANDARD TUBE AGGLUTINATION TEST |
| RBPT | - | ROSE BENGAL PLATE TEST |
| MAT | - | MICROAGGLUTINATION TEST |
| 2-ME | - | MERCAPTOETHANOL AGGLUTINATION TEST |
| ELISA | - | ENZYME LINKED IMMUNO SORBENT ASSAY |
| PCR | - | POLYMERASECHAIN REACTION |
| PUO | - | PYREXIA OF UNKNOWN ORIGIN |
| LPS | - | LIPOPOLY SACCHARIDES |
| S-LPS | - | SMOOTH LIPOPOLYSACCHARIDE |
| CSF | - | CEREBRO SPINAL FLUID |

# CONTENTS

# Seroprevalence of brucellosis among animal handlers and analysis of risk factors

## 1.Introduction

Brucellosis is a re-emerging infectious disease, by *Brucella spp* and usually transmitted to humans from infected animals. It is a most important source of disease in humans and live stocks. The clinical manifestations in humans differs from an acute febrile illness to a chronic, low grade ill defined disease. In each year 5,00,000 new cases are reported worldwide, but according to the WHO these numbers greatly underestimate the true incidence, because the clinical picture of human brucellosis is extremely variable and misdiagnosed by physicians. However, the actual incidence seems to be twenty five times higher than the reported incidence. *Brucella* is considered a biological weapon in the category B pathogen, inhalation of only a few organisms is sufficient to cause infection.

### 1.1 History

Since, the man started to domesticating the livestocks brucellosis has emerged as a disease.[1] The disease was characterized with fever which has remissions and intermissions. It was known by several names in relation with places where it was more common viz Malta, Mediterranean, Gibraltar and undulant fever. [2]

In 1895, J.A. Marston have given a correct description of the disease and he named it as “Mediterranean” or “Gartnic remittent fever”. The cause of the disease was obscure until 1886. Sir David Bruce a British army Medical Officer in 1887 demonstrated a plenty of microorganism from a spleen of military personals who had infected and died of Gibraltar fever. Bruce reproduced the disease in chimpanzees with the isolates, and named this organism as “ Micrococcus melitensis.”[3]

In the year 1897, Huges named the fever as undulant fever. Bang isolated the bacilli from a aborted products of animals and named that Bacillus abortus. [4] On June 14, 1905, Zammit isolated the bacilli from infected goat’s urine and milk . Zammit also developed an agglutination test to detect agglutinin in the milk. Further work rendered it evident that goat was the natural host of Micrococcus melitensis and that infection was communicated to man by consumption of raw milk.[5]

Alice C. Evans (1918) of Washington D.C., drew attention to the similarity between Micrococcus melitensis and Bacillus abortus, the causative agent of Malta fever and infectious miscarriages in cattles.[6] In 1920, the generic name *Brucella* was proposed by Meyer and Shaw for this microorganisms in honour of Bruce.[7] Subsequently a 3rd species, *Brucella suis* was identified as a cause of epizootic abortion of swine. 4 th species, *Brucella canis* was identified as the cause of abortion in beagles. *Brucella ovis* was identified as the causative agent of contagious

epididymitis of rams and *Brucella neotomae* was isolated from desert wood rats. Out of these the most important species that infect human beings are *B. melitensis, B. abortus, B. suis* and *B. canis*.[2]

## 1.2 Epidemiology

### 1.2.1 Global scenario

Brucellosis continues to be of great health significance and economic importance in many countries. Countries like the Arabian Gulf, the Indian subcontinent, Mediterranean basin and parts of Mexico as well as central and South America are especially endemic for human brucellosis. In endemic areas, the reported incidence of human brucellosis varies from <0.01 to > 200 in one lakh population.[1] The world burden of human brucellosis remains to be > five lakh infections per year.[8] In 1990, Egypt and many Arabian countries reported a incidence of > 90 thousand cases of human brucellosis per year.[9] Every year, in USA 100 - 200 *brucella* cases are reported. The incidence and prevalence of brucellosis varies from country to country. In endemic areas only few cases are reported it reflects the poor surveillance and under reporting.[10]

### 1.2.2 Indian scenario

In India, brucellosis is a major emerging veterinary and civic health concern. The existence of brucellosis was noted in the 20$^{th}$ century itself from then it has been reported from almost all states of India but the situation varies greatly between states.[11] India is an agricultural country

and more than 3/4 th of the population resides in rural areas having close contact with wild and domestic animals. Thus, the Indian population stands at a significant risk of acquiring zoonotic diseases as well as brucellosis. On the other hand, the reports regarding the distribution of brucellosis are often incomplete. This may be due to the fact that poor diagnostic services, decreased awareness and no exchange of data among health and veterinary authorities.[1]

### 1.3 Agent

*B.melitensis*, *B.abortus, B.canis and B.suis* are the causative agent of human brucellosis. The species level identification of the bacteria are essential because the severity of disease in humans is mainly dependent on the type and source of the infection. Human brucellosis commonly caused by *B.melitensis* and *B. abortus* among this *B.melitensis* produce severe disease. The other species in this genus also cause disease in humans.[12]

### 1.4 Morphology

*Brucella* are small gram negative coccobacilli. They are shorter and slender have straight axis. The length vary from 0.6 - 1.5µm and breadth is 0.5 - 0.7µm. They are arranged singly, pairs, short chains or in small groups.[2]

*Brucella* are gram negative, weakly acid fast, non-motile and non-sporing. Resists the decolourisation by acid and alkali and used in staining of infected tissues. A modification of Ziehl Neelsen method may be used, though it is not absolutely specific for *Brucella.*[2]

**1.5 Genome**

*Brucella* genome has 2 circular chromosomes of 2.1 Mb and 1.5 Mb except *B.suis* biovar 3, which contains one chromosome of 3.1 Mb. The replicons code for important metabolic and replicative functions.[13] Natural plasmids have not been detected in *Brucella*, although transformation has been effected by wide host range plasmids after conjugative transfer or electroporation.[14]

**1.6 Antigenic determinants**

The important antigens of *Brucella* includes the lipopolysaccharide (LPS) complex and two related polysaccharides. There are at least two antigenic determinants A (abortus) and M (melitensis ) which have been identified as the O chain of the LPS complexes. Strains of *B. abortus*, *B. melitensis* and *B. suis* can be A, M or A and M antigen positive. S-LPS is the immune predominant and is one of the important virulence marker. Plenty of periplasmic, outer or inner, cytoplasmic proteins are identified.[1]

## 1.7 Cultural Characteristics

*Brucella* is one of the difficult organisms to cultivate. They are aerobic bacteria and fail to grow in strict anaerobic conditions. Br. abortus is capnophilic in nature. This should be incubated an atmosphere of 5% to 10% $CO_2$ at 35°C to 37°C and colonies usually appear within 4 to 5 days, but cultures should be kept for one month before they are declared as negative. [2]

*Brucella* species are usually isolated from blood or bone marrow cultures during the acute illness. Eduardo Gotuzzo *et al* [15] reported that culture from bone marrow yields better results than blood cultures. The prior use of antimicrobials will reduce the blood culture positivity but will not affect the bone marrow culture. We can isolate the organisms from samples like CSF, lymph, urine and liver of infected patients.

The biphasic culture method by Castaneda is recommended for the culture of blood and other body fluids.[16] Solid media recommended for isolation of *Brucella* are trypticase soy agar, serum dextrose agar, tryptose agar, Douglas agar and *Brucella* agar. The aqueous phase consists of the same basal medium without agar.[2]

*Brucella* strains grow on chocolate agar, blood agar and *Brucella* agar or some infusion base agar. 5% heated horse or rabbit serum

enhances growth on all media. The optimum pH range is 6.6 to 7.4. Temperature range is 20°C-40°C optimum being 37°C.[2]

On subculture to solid media the colonies appear after 4-5 days and are 0.5 to 1 mm in diameter, raised convex, circular, moist, translucent and easily emulsifiable. On further incubation they increase in size to 4-6 $mm^2$.

In synthetic media, Pantothenate and erythritol improves the growth of *brucella* and tryptophan and cystine are highly toxic to Br. abortus[17].

**1.8 Biochemical Reaction**

*Brucella* produces catalase and many species of *Brucella* are oxidase positive except Br. ovis and *B. neotomae*. Many species reduces nitrate and it is variable in *B.ovis* and *B. suis* biogroup. Almost all species, except *B. ovis* and some strains of *B.melitensis* and *B. abortus* hydrolyze urea rapidly. *B. ovis* may hydrolyze urea weakly or not at all.[2]

*B. abortus* produces moderate amount of $H_2S$ for 4-8 days except the Danish variety, which does not produce $H_2S$. The American strains of *B. suis* and *B. neotomae* also produce $H_2S$, while *B. melitensis*, *B. canis* and *B. ovis* do not produce $H_2S$. *Brucella* are negative for MR, VP and do not produce Indole.[2]

The commonly used method to identify *Brucella* from humans is the dye inhibition test. Dilutions of basic fuchsin and thionin are put up. *B. abortus* should grow only on basic fuchsin, *B. suis* only on thionin and *B. melitensis* on both[2].

The $CO_2$ requirement of freshly isolated *B.abortus* for growth helps in differentiating them. The use of monospecific sera against the species helps in differentiating them from each other.

**1.9 Susceptibility to Physical and Chemical Agents**

*Brucella* are destroyed by heat at 60°C in 10 minutes and by 1% phenol in 15 minutes. They are killed by pasteurization. They remain viable for 10 days in refrigerated milk, 30 days in ice cream, 120 days in butter and for varying period in cheese depending on its pH. They are sensitive to direct sunlight and acid and tend to die in butter milk.

*Brucella* will survive in bovine feaces for four months, in uterine exudate for at least 200 days and in liquid manuer for upto 2 ½ years if the temperature is kept near 0°C. Formaldehyde is the most effective of the commonly available disinfectants. [2]

**1.10 Susceptibility to antimicrobial agents**

Most *Brucella* strains are sensitive to gentamicin, kanamycin tobramycin, amikacin and streptomycin. In vitro they are insensitive to

β-lactam antibiotics except monolactum and clavalunate potentiated amoxicillin. Sensitivity to tetracyclines is almost universal. [2]

### 1.11 Pathogenesis

*Brucella* enters into the human tissue by various routes like abrasions in the skin, alimentary canal, respiratory route.[18] At or near the site of entry the bacteria are likely to be engulfed by either mononuclear or polymorpho nuclear cells. After their entry into the macrophages, they are transported into various organs of the body.

Within the phagocytic cells, these organism resists the phagosome, lysosome fusion and undergo replication[19]. As a result of this, the infected cells are destroyed by the pathogen if the process is not checked by the immune system.

### 1.12 Host immune response

Infection with *Brucella* usually results in the induction of both humoral and T-cell mediated immune response.

### 1.13 High risk groups

Veterinarians, abattoir worker, cattle ranchers, dairy farmers, meat sellers, meat inspectors, shepherds, goatherds, hunters, lab workers, travellers and persons consuming raw dairy products are particularly at risk.

## 1.14 Transmission

The mode of transmission and prevalence of brucellosis depend on many factors like dietary practice, customs in the society, socioeconomic status, climate, animal husbandry practices and environmental sanitation.

The species of *Brucella* that are infective to humans and livestock reservoir includes, *B. melitensis* in goat and sheep, *B. abortus* in cattle, *B. suis* in pigs, and *B. canis* in dogs. The major source for transmission of disease in general population is the consumption of unpasteurized milk and its products which contains high amount of organism[1].

Though animal muscles contain low bacterial load, eating of improperly cooked meat can leads to brucellosis. Handling of live cultures and travel to endemic area also increases the risk.[1]

Recently other routes of transmission have been identified like, infection through breast milk,[20] sexual transmission,[21] blood transfusion, bone marrow transplantation, infection contracted by an obstetrician during the delivery of a transplacentally infected baby[21] and accidental inoculation with animal vaccines *B. abortus* strain S19 and *B. melitensis* Rev-1.

## 1.15 Clinical spectrum

Human brucellosis is a systemic infection, presents with wide spectrum of clinical manifestations. It usually presents as a fever without any apparent focus of infection. But in few cases focal forms are seen

which might affects many organs and systems of the body with the skeletal forms being more common, cardiac and neurological forms being more severe. The case fatality rate may be up to 2% and usually results from endocarditis. [2]

The onset of the disease may be either acute or insidious. The infective dose is low. It has a incubation period of 10-21 days to several months. Acute brucellosis mostly presents as a undulant pattern of fever with profuse sweating, body pain, loss of appetite and weight loss and fatigue. Liver, spleen and lymphnodes become enlarged.[22]

### 1.16 Complications

Osteoarticular, alimentary canal and hematologic forms are the common complications but the cardiac, neurologic forms are more severe but infrequent.[45]

### 1.16.1 Skeletal complications

Although monoarticular septic arthritis occur, 30 to 40% of patients have reactive symmetrical polyarthritis involving the knee, hip, shoulder, sacroiliac and sternoclavicular joints. Cultures of synovial fluid are positive in about 50% of cases.[24] *Brucella* osteomyelitis is rare.[25] Bursitis and tendinitis due to *Brucella* have also been reported but is very rare.

### 1.16.2 Gastrointestinal system

Gastrointestinal manifestations of *Brucella* infection are generally mild and may include nausea, vomiting, constipation, acute abdominal pain and diarrhea. Hepatic and spleenic enlargement may be seen in 15-20 % of cases and abscesses may develop in the liver and spleen. Mild jaundice is seen in few cases.[25]

### 1.16.3 Haematologic complications

The heamatologic manifestations of brucellosis include anemia. leucopenia, thrombocytopenia and clotting disorders.[26] Granulomas are found in the bone marrow in up to 75% of cases, but they are small and indistinct.

### 1.16.4 Cardiovascular system

Cardiovascular complication of Brucellosis include endocarditis, myocarditis, pericarditis, aortic root abscess, mycotic aneurysms, thrombophlebitis with pulmonary aneurysms and pulmonary embolism. *Brucella* endocarditis usually involve the valves. It is reported to be the most common cause in fatal cases of human brucellosis.[25]

### 1.16.5 Central nervous system

Neurobrucellosis is uncommon but serious and includes meningo encephalitis, multiple cerebral or cerebellar abscesses, ruptured mycotic

aneurysms, myelitis, GB syndrome, cranial nerve lesion, hemiplegia, sciatica, myositis and rhabdomyolysis.[25]

### 1.16.6 Genito urinary system

The genitourinary infections due to *Brucella* include epididymo-orchitis, prostatitis, seminal vesiculitis, dysmenorrhoea, tubo-ovarian abscess, salpingitis, cervicitis and acute pyelonephritis. *Brucella* has been isolated from urine in up to 50% of cases of genitourinary *Brucella* infection.[25]

### 1.16.7 Ocular Complication

A variety of ocular complications have been reported in patients with brucellosis. Uveitis is generally a late manifestation consisting of chronic iridocyclitis, nummular keratitis, multifocal choroidits and optic neuritis[25].

### 1.16.8 Skin Manifestation

Skin manifestation of *Brucella* are uncommon. They include maculopapular eruption, purpura and petechiae, multiple cutaneous and subcutaneous abscess, discharging sinuses, superficial trombophlebitis, erythema nodosum and pemphigus.[25]

### 1.16.8 Respiratory System

Respiratory illness due to *Brucella* include sore throat, tonsillitis, dry cough, hilar and paratracheal lymphadenopathy, pneumonia, solitary or multiple pulmonary nodular lung abscesses and empyema.[25]

### 1.17 Chronic Brucellosis

Since the onset of symptoms of brucellosis is insidious, not easy to differentiate between acute and chronic form of the disease. Most patients with chronic brucellosis have persisting foci of infection, such as suppurative lesions in bone, liver or spleen. Some patients who have had brucellosis will continue to have symptoms like malaise, lassitude and depression in the absence of objective evidence of infection.[27]

### 1.18 Laboratory diagnosis

The clinical presentation of brucellosis mimics some infectious and noninfectious conditions. So, the diagnosis of the disease is very difficult and frequently delayed or missed. Early diagnosis of the disease and inclusion of proper antimicrobial treatment is vital for patients, especially to prevent the development of complications and appearance of relapses.

### 1.18.1 Culture

Definitive diagnosis of brucellosis is made when *Brucella* are recovered from blood, bone marrow, lymph nodes, cerebrospinal fluid, urine, pus from abscesses and rarely from sputum, breast milk, vaginal discharges and seminal fluids. Failure to isolate the bacteria is a drawback of blood culture due to the fact for isolation, the bacteria needs extended incubation period, special media, and frequent subcultures are due to the fastidious, slow growing nature of the bacteria. Though bone marrow cultures are said to be the gold standard one, but results have not been generally reproducible. In acute stage of the disease blood culture has increased sensitivity. Inspite of this, this test is not done routinely in rural areas, where the disease is endemic.[28]

### 1.18.2 Serology

The limitations of a blood culture makes serology the most useful way to diagnose. Detection of antibodies directed against the S-LPS, internal cytosolic proteins are the important antigens which are used in the serological diagnosis of brucellosis. IgM agglutinins start to appear in the blood after 7 days of disease followed by IgG. This IgG agglutinins will persist for longer periods even after the disease has been cured.[29]

Many serological tests have been used for the diagnosis of human brucellosis.The test that are presently available are standard tube agglutination test (STAT), Antihuman globulin test, Rose bengal plate

test (RBPT), complement fixation, Enzyme linked immunosorbent assay (ELISA) and Immuno fluorescent antibody assay. STAT, Coombs test, ELISA are the widely used method for the detection of *Brucella* agglutinins.[29]

#### 1.18.2.1 Antigen detection

Antigen detection which is the suitable alternative to blood culture but this test have not been standardized yet.[30]

#### 1.18.2.2 Antibody detection

**a. Rose Bengal Plate Test (RBPT)**

This is a simple and rapid plate agglutination test and has a higher degree of sensitivity for the diagnosis brucellosis irrespective of the stage of disease. This high sensitivity, rapid and easy to perform makes the test ideal for screening the patients for human brucellosis.[1]

**b. Standard tube agglutination test (STAT)**

STAT is the accepted test for obtaining quantitative information about the immune response against specific brucellar antigens. This test estimates the quantity of agglutinating antibodies (IgG and IgM). When suitable clinical manifestations are present a presumptive diagnosis of brucellosis is made serologically as a titre of 1in 160 and above. Where in endemic areas, a titre of 1in 320 dilution makes this test more specific one.[1]

Even though this test being the more standard one, it is time-consuming hence, unsuitable as a primary test for laboratories with large specimen workloads.[31] In some sera a blocking factor may interfere with agglutination at low serum dilutions (Prozone phenomenon) this may be due to the presence of IgA or other non-agglutinating antibody. Another drawback is, the diagnosis of brucellosis cannot be established on the antibody titer by these classic test alone, because healthy persons engaged with animal husbandry practices may show significant titers of *Brucella* agglutinins.[32] The other disadvantage is cross reactions between *Brucella* and other bacteria such as *Vibrio cholerae*, *Pseudomonas maltophilia*, *Francisella tularensis, Yersinia enterocolitica and Escherichia coli O: 157*. This reactions can result in false positives in the serologic tests for brucellosis. [2]

**c. Mercaptoethanol (ME) agglutination test**

This test determines the nature of immunoglobulins responsible for the agglutination in the STAT. In STAT, agglutination may be due to IgG, IgM or both. 2-mercaptoethanol destroys the agglutinating activity of IgM and therefore agglutination in this test is indicative of IgG. This 2-ME dissolves the disulphide links of IgM pentamer, thus interfers with its agglutinating capacity while not affecting IgG antibodies.[33]

**d. Microagglutination test (MAT)**

Microagglutination test is a simpler and more efficient test than STAT. It can be performed more rapidly and employs less serum and antigen. It has been found to be more sensitive and specific than STAT. Since the MAT is simpler to perform than STAT and can potentially be automated. [34]

**e. Anti human globulin test (Coombs test)**

This test detects the incomplete antibodies. Nowadays, the Coombs test is rarely performed in routine clinical laboratories because the procedure is too complex, time consuming and labor intensive, requires skilled persons to perfom the test. [1]

**f. Complement Fixation Test:**

This test is mainly used in the diagnosis of chronic brucellosis. [3] It detects the complement fixing antibodies. It is the IgG antibody in brucellosis, which readily fixes complement. IgG appears late in the disease and hence this test is more useful in diagnosing chronic brucellosis. [33]

**g. Enzyme Linked ImmunoSorbent Assay (ELISA)**

ELISA which is a more sensitive and specific test with increased performance than other conventional tests. It gives a profile of *Brucella* specific IgA, IgM and IgG agglutinins in case of acute and chronic brucellosis.[35]

ELISA gives high sensitivity and specificity when compared to STAT. [31]and it is used for the diagnosis of chronic and complicated cases. In endemic areas this test has significant diagnostic advantage than other conventional methods.[35] ELISA can be used to study the subclass of Ig, so that the role of each Ig in the different phases and evolutional forms of brucellosis can be ascertained.[29] It detects the antibodies against many bacterial antigenic structure like S-LPS or cytoplasmic protein antigens.[36] Applying a combination of IgM and IgG ELISA testing could be of value for the definitive diagnosis of brucellosis in developing countries, where diagnostic capabilities for culture, including automated culture systems and Polymerase chain reaction are poor.[31]

**h. Immunocapture agglutination test**

The Immunocapture agglutination test has high sensitivity and specificity that detects the antibodies against especially for S-LPS. [37]

### 1.18.3 Genomic detection

Molecular diagnosis by polymerase chain reaction (PCR) has been used for confirmation and differentiation of *Brucella*. This methods are very useful for follow up testing of unusual phenotypes. PCR is a very expensive test and not used routinely as a diagnostic method.[1]

### 1.18.4 Newer rapid tests

The latex agglutination test and lateral flow immunoassay are the newer tests available for the detection of *Brucella* IgG and IgM. It is a

rapid and simple test with increased specificity and sensitivity and is a ideal test can be used in remote area.[38]

In this back ground, the present study was conducted to assess the seroprevalence of brucellosis among animal handlers and to analyse the risk factors associated with this infection in Tirunelveli district of Tamil nadu.

# 2. AIMS AND OBJECTIVES

1. To detect the seroprevalence of brucellosis among animal handlers in Tirunelveli district of Tamil nadu.
2. To analyse the risk factors associated with the seropositivity of *Brucella* infection.

# 3. REVIEW OF LITERATURE

Brucellosis is one of the world's major zoonotic disease. It has a variable trend in United states and the European countries but has a predominant presence in Asia and in developing countries. It is a unrestrained public health crisis in developing countries like India, here it is a common but often a ignored disease and it is also a disease of considerable economic and social importance. The prevalence of human brucellosis is difficult to estimate since many times the disease has been misdiagnosed or undiagnosed because of their inapparent or protean manifestations. Nowadays a wide battery of serological tests are available for the diagnosis of human brucellosis.

## 3.1 Prevalence

### 3.1.1 Global prevalence

Brucellosis is an important global problem. It is a reemerging zoonotic disease cause severe economic loss and infection to humans. Most of the developing countries the burden of the disease is increased by the absence of national surveillance programme and inadequate laboratory facilities. Some countries, conventionally considered to be endemic-e.g: Israel, France and most of Latin America but now controlled the brucellosis.[1] But, in central Asia the new foci of infection been emerged. The circumstances in Syria has been fast declining.

In Arabian countries, the prevalence of human brucellosis is higher as a result of increased live stock production units.

In 1999, Mohammed A Al Sekait [39] done a seroepidemiological survey in Saudi Arabia and the results revealed that the seroprevalence was 15.0%. Seropositivity of brucellosis from various regions of Mediterranean basin varied from 8% in Jordan [40] to 12% in Lebanon and Kuwait.[41,42] Even higher seroprevalence rates have been reported in Sub-Saharan countries, with 18% in Uganda [43] and 13% in Nigeria.

A Seroepidemiological study conducted in Kars district of Turkey for period of 2 years from Jan- 2004 to Dec-2006 revealed that the seroprevalence was 17.88%. [44] In a study conducted by Apan *et al* 2007 [45] in Middle Anatolia, Turkey, the seroprevalence of brucellosis was determined as 3.2%.

Kose *et al* in 2006 [46] reported the seroprevalence of 2.9 to 8.5% in rural and suburban communities in West Anatolia, Turkey. Another study performed in Middle Anatolia by Cetinkaya *et al* 2005 [47] revealed that the seropositivity was 4.8%.

A Serological survey conducted by Cadmus S.I.B *et al* 2004 [48] showed that the overall seroprevalence of brucellosis in Ibadan , Southwestern Nigeria was 31.82%.

Hajia *et al* [49] had done a study to estimate the antibody levels of Brucellosis and showed the prevalence rate to be 3.28% in Hamedan,

Western Iran. Bokaei *et al* [50] studied the prevalence rate of brucellosis in Birjand, Iran. It was 37 in one lakh population

A study conducted on the seroprevalence of brucellosis in 184 suspected cases [51] for period of 2004 to 2009 showed that 5.4% were seropositive for *Brucella* agglutinins in their serum.

A study done in Pyrexia of Unknown Origin patients of Makurdi in 2004 [52] revealed that among a total of 1040 serum samples screened, the overall seroprevalence was 7.6%.

### 3.1.2 India

India is an agricultural country and majority of people in our country are engaged in agriculture related activities like seasonal agriculture labours, dairy product selling and animal meat selling. Several studies and publications from India revealed that human brucellosis is a common disease. Studies conducted from various centers of India have reported seroprevalence ranging from 0.8 to 6.8% in patients with PUO.[53]

A prospective study conducted in North India by Handa R *et al* [54] observed that 3.3% patients with PUO had acute brucellosis while 6.6% had serological evidence of previous *Brucella* infection. 14% of the asymptomatic, 'at risk' individuals screened were seropositive for *Brucella* infection.

Kadri SM *et al* [55] in 2000 observed that out of a 3,532 hospitalised patients for PUO 28 (0.8%) were found seropositive for brucellosis .

A study done by Appanavar SB *et al* [56] in North India revealed that the seroprevalence of brucellosis in PUO cases were 9.94% of which 45% had acute infection and the remaining 55% had chronic infection.

Moti Yohannes *et al* [57] in 2011 had done a seroepidemiological survey of human brucellosis in and around Luthiana among 241 high risk persons with and without pyrexia of unknown origin and the seroprevalence documented was 26.6%. In Orissa it was 6.8% [58] and in Andhra Pradesh it was 11.51%.

A study conducted by Vaishnavi C *et a l* [59] reported that out of 292 serum samples of blood donors, 16.8% had positive *Brucella* antibodies of this 0.36% had high titres of antibodies.

A Seroprevalence study conducted in Davangere, Karnataka[60] reported that the overall seroprevalence of brucellosis was 3.3%. It was 2.4% in general population and 11.1% in veterinary staff. In study from Kerala in 2005 [61] reported that the overall seroprevalence of brucellosis was 1.6%. Among the general population the prevalence rate was 2.45% and in veterinary students it was 1.14%.

In Gujarat[62] 8.5% prevalence of 8.5%, in Belgaum [63] seroprevalence of 8.5% in publics and 5.8 to 14.3% in veterinary persons. In another study in 2006, reported a prevalence rate of 1.8% in Bijapur.[53]

Panjarathinam *et al* [64] reported the seropositivity of 6.5% in aborted women. The high incidence of spontaneous abortion may be due to the fact that this organism infects the chorio amniotic tissue

Persistence of infection in animal reservoir, low physician awareness, poor availability of diagnostic facilities, and the non existence of regional data bases contribute towards the perpetuation of this zoonosis in India, while it has been eradicated from most developed countries.

**3.2 Age**

Brucellosis affects all age groups.

In a study, Fatima *et al* [65] observed that those who were in the age of 51 to 60 yrs had increasd seropositivity followed by of 41 to 50 yrs. As brucellosis is an occupational disease, individuals in this age were at a greater risk because of prolonged years of exposure.

In a study by Ramos TRR in 2006 [66] reported that there was a significant association between age group and seropositivity for this disease, with individuals above 40 years of age more predisposed to being infected, which is similar to the findings of Bigler *et al* [67] and Feliciano *et al* reported that there is increased frequency of seropositivity with advancing age, probably due to a longer period of exposure to the organism.

A study from Karnataka, in 2007 [68] noted that the highest prevalence of 45.36% was found in 41to50 yrs of age followed by

32.98% in the age of 31 to 40 yrs. 14.43% were in the age of 51 to 60 yrs. 7.21% cases in the age of 21 to 30 yrs. This study reported that a wide variation in the age group of seropositives.

Jama'ayah MZ *et al* in 2011[51] found in their study that persons were in the age of 20 to 45 yrs had higher seroprevalence. 70% were in the age of > 40 yrs and 2% were < 20 yrs of age.

Mahmoud N Abo-Shehada *et al* in 1996 [69]observed in their study that seroprevalence of brucellosis increased with age. Among 26.3 % of seropositive veterinary surgeons the highest prevalence was noted in 34-43 years of age .

Metri Basavaraj C *et al* in 2011[60] reported that in veterinary surgeons, brucellosis was more common among in the age group of 31-40 years followed by persons more than 41 years. Randhawa *et al* [70] have reported that individuals in the age of thirty (14.2%) and forty years (7.0%) had the highest prevalence among high risk persons. More prevalence in this age group may be due to increased activities with regards to their occupation, thereby increasing the risk of acquiring the infection.

Ali *et al* [71] reported that those were in the age of 25 to 35 yrs had higher soprevalence. Kadri *et al* [55] also observed that persons of 21 to 30 yrs had the higher (43%) seropositivity.

A seroepidemiological study done in 1999 [39] revealed that seropositivity of brucellosis increases with age advances. The lower seroprevalence rates found in paediatrics may be due to the fact that adults had the much exposure to animals. A study by Abu-Shehada *et al*[69] and Araj GF *et al* [41] reported an increase in frequency of seropositivity when the age advances.

Ofukwu et al in 2004 [52] in their study reported that higher seroprevalence (38%) were in the age of 21 to 40 yrs and 32.9% in the age of 41to 60 yrs. 13.9% and 15.2% were in the age group of 0- 20 years and in the age group of 60 and above years. There was no significant association in the age specific prevalence rates.

**3.3 Sex**

Brucella affects both males and females equally. There is no sex wise discrimination between the two sexes provided they are equally exposed to the potential risk factors. But most of the studies reported that males are most commonly affected than females which is perhaps justified by the greater presence of men in the veterinary profession, slaughterhouses and dairy farms.

A study from Malasyia [51] showed that seropositivity was common in (90%) males than (10%) females.

Moti Yohannes *et al* [57] observed in their study that all seropositive cases were men. Various other studies conducted by other researchers

also found that males were more commonly affected than females. This is due to the fact that males are employed in field of animal husbandry or are exposed to infected animals and their secretions.

Kapoor *et al* in 198 [72] and Hussein *et al* in 2005 [73] reported higher seropositivity among females.

A study from Nigeria,[52] reported that out of 79 *Brucella* positive cases, 45.6% were male and 54.4% were females. This results were in concordance with the findings of Falade and Junaidu *et* al. [74]

Aseroepidemiological survey of brucellosis among animal handlers [38] revealed that seropositivity and gender was not significantly associated.[75] A study by Al seikait [39] also reported that there was no significant difference between the sexes.

A study by Ramos TRR in 2005 [66] found that among the 26 serology positive individuals 3 (2.0%) were females and 23 (4.7%) were males. There was no significant association between sex and seropositivity in the individuals studied.

### 3.4 Education

Low literacy is one of the risk factor for brucellosis because they are not aware of the zoonotic diseases and they don't follow any hygienic practices after handling the animals and their products. Karimi *et al*[76] reported that significant association between low literacy and seropositivity.

Sumer *et al* [77] and Fatima *et al* [65] found that no association between the literacy and seropositivity.

### 3.5 Residential Background

A study conducted in Lahore [65] reported that out of the 78 seropositive individuals , 22.6% were from urban localities and 20.3% from rural areas. The residential background was not significantly associated with seropositivity. Baba's *et al* [78] also reported the similar findings.

Nabi *et al* [79] reported that 84.2% of seropositives reside in rural area and 15.8% from urban areas. The increased prevalence in rural people may be due to the fact that they are involved in cattle rearing and handling.[68]

In a study from different regions of Saudi Arabia,[39] the seropositivity was higher ( 26.2%) in those from rural areas compared with (9.5%) in urban areas. This may be due to the fact that ingestion of raw milk and their products from infected animals as well as handling of aborted fetuses, placenta and uterus and vaginal secretions may be considered sources of infection for the high positivity of brucellosis among the rural population.

### 3.6 SocioEconomic status

Al sekait [39] noted the highest (24.4%) seroprevalence among the persons of low socioeconomic status when compared to the persons (6.3%) of high socioeconomic status.

### 3.7 Occupation

Meky *et al* reported that workers in occupations dealing with animals had a 2.4-times higher risk of acquiring the infection than those in occupation not dealing with animals.

*Brucella* are detected in the abortive products and carcasses of infected animals and their secretions thus brucellosis becomes an occupational disease for veterinary persons, slaughterhouse workers and farmers.[66]

Brucellosis is an occupational hazard to veterinary professionals. They are exposed to all risks except their awareness about the zoonotic disease. These persons usually acquire the disease by cuts in the skin and conjunctival splashes or through inadvertent injection of animal vaccine and during the removal of infected uterine products.[1]

A study conducted in high risk group individuals observed that the seroprevalence of brucellosis was 41.2% in Live stock inspectors, 30.9% in veterinary assistants, 12.37% in veterinary doctors, 6.18% in supervisors and in sweepers, 2.06%, 1.03% in shepherd and butchers respectively.[68]

In study by Thakur [80] reported the overall seroprevalence of 4.97% among animal handlers of which veterinary surgeons (17.39%) had the higher seroprevalence.

Mrunalini *et al* [81] reported seroprevalence of 25.24% in veterinarians, 23.3% in para-veterinarians, 12.62% in farmers, 11.65% in shepherds and 6.8% in other occupational groups.

A study conducted in the year of 2003 [82] among animal handlers found that out of 225, 5.33% were seropositive. Among them 14.63% of veterinary doctors and 4.51 % of dairy farms workers were seropositive.

A seroprevalence study on persons of high risk occupation revealed that the veterinarians (50%) had the highest percentages of seroprevalence followed by farmers who had a seroprevalence of 23.9 % and butchers had 9.2% prevalence rate.[41] In Saudi Arabia, 7.14% of veterinary surgeons and 2 .67 % of butchers were positive for *Brucella* agglutinins.

Dairy workers are exposed continuously to the pathogenic agent while milking the animals because their hands get contaminated.[82]

Eritrea, Omer *et al* reported [83] that the highest prevalence among dairy farm workers 7.1% and owners, followed by 4.5% in veterinary staffs. Soman and Kothari reported that 4% of dairy workers were

positive for *Brucella* antibodies. Sohaila *et al* reported the seroprevalence of 6.1% among dairy workers.

Meat handlers, abattoires are in direct contact with carcasses of infected animals and raw meat are at greater risk. They are infected through abrasions and cuts in mucous membrane and splashes in conjunctival mucosa.

Cadmus S.I.B *et al* 2006 [48] found that the overall seroprevalence of brucellosis was 31.82%. Of this 63.63% were butchers. Butchers are frequently infected might be due to the fact that they do not use PPE and exposed to infective material like blood, vaginal secretion, retained uterine products and urine from infected animals.

A study by Kumar et al among abattoir workers [84] in which, blood collectors had the highest (99.77%) seropositivity followed by, 68.96% among animal handlers, 68.00% in butchers, 57.14% in sweepers and 28.57% were among the veterinary surgeons.

A study conducted in 2004 [52] revealed that the seroprevalence of 43.8% in abattoir workers/ butchers. This was similar to the reports by Falade 1974, Ocholi 1993, Edu, 2005 the prevalence was 28 to 57% among abattoire workers .

A study on seroepidemiological survey of brucellosis antibodies from various occupational groups in Saudi Arabia [39] stated that the

seroprevalence of was higher in occupationally exposed group such as meat sellers, veterinary professional and farmers.

In Pakistan, Masoumi *et al*[85] carried a study among abbatoire workers and found the seroprevalence to be 8.33%. In Algeria, the seroprevalence was found to be 37.6% among high risk persons.

Shepherds also at the risk of acquiring the disease due to the widespread infection of *Brucella melitensis* in sheep.

Sonmez et a observed the seroprevalence of brucellosis to be 6.2% among farmers in East Anatolia of Turkey. MH Salari *et al* in 1997 [75] reported a seroprevalence of 3.75% among animal farmers of Yazd province of central Iran. The poor hygienic practice favours the transmission of infection among farmers

A seoprevalence study done in high risk group showed that significantly higher seropositivity of 8.2% among high risk groups.[64]

Asanda [86] reported that seropositivity was increased in persons who are involved in livestock and their product activities than those in other productive activity.[39] A study of Detection of *Brucella abortus* antibodies in animal handlers in Pune by S Mudaliar et al 2003 [82] found that out of 225 animal handlers screened, 15.33% were positive for *Brucella* antibodies.

### 3.8 Duration of work

A study from Northern Jordan observed that the seropositivity of brucellosis was significantly high in persons those who are occupationally exposed to animals for more than 22 yrs when compared to people having work of less than 22 years.[69]

Karimi *et al* [76] and Sohaila *et al* reported strong correlation between the seropositivity and duration of exposure to animals.

### 3.9 Other Risk factors

*Brucella* infection is transmitted to humans through the consumption of raw milk or unheattreated dairy products.

Hasanjani Roushan etal [2] and Al-Fadhli *et ai* Observed that consumption of fresh cheese, unpasturised milk as the risk factor.[39]

Ali *et al* [71]and Al Sekait [39] reported contact with animals, raising animals in the vicinity of residence and drinking unpasteurised milk as risk factors.

### 3.10 Vaccination

Animal vaccines are being used with increasing frequency to protect the health of live stocks. However, the persons who are handling the vaccines get exposed by means of inadvertent inoculation or by other routes. During the vaccination of animals accidental human inoculations can occur. Studies reported that there are few reports of adverse events in persons associated with the use of animal vaccine.

The attenuated strain of *B. abortus* S19 is the vaccine commonly used to prevent animal brucellosis. Nowadays rough mutant strain RB51, replaces the S19 vaccine because it is less virulent and will not interfere with serological tests. Though they are less virulent, both are infectious to humans. Human get infected through conjunctival splashes, cuts in skin and mucous membrane or via infectious aerosols.

J. C.Wallach [87] conducted a survey to evaluate the consequences of exposure to the vaccine strain Br. abortus S19 from employees of vaccine-manufacturing plants in Argentina and the results revealed that active infection was observed in 30% of individuals, out of which 5 (23.8 %) were given a history of accidental exposure to the vaccine.

Ashford DA *et al* [88] reported in their study that 81% of individuals got a accidental exposure to RB51 vaccine by needle stick injury, 15% by conjunctival splash, and 4% by cuts in skin and mucous membrane among the persons who handled the live cultures or participated in the vaccination of animals.

A seroepidemiological study conducted by Moti Yohannes *et al* [57] reported that among the veterinarians, seroprevalence of brucellosis was significantly high in those who participated in vaccination of animals against brucellosis than those who did not.

### 3.11 Diagnosis

The heterogenous type of clinical symptoms, paucity of clinical signs and the presence of subclincal and atypical infections makes the clinical diagnosis difficult. Isolation of bacteria and the serological evidence of infection are vital to confirm the clinical diagnosis.

In acute stage of disease, IgM agglutinins starts to appear within a few days of infection and the peak level is reached after one month. IgG antibodies may be detected after 10 days and it attains the peak level 8 weeks after the infection. In acute stage of the disease The IgM level always exceeds the IgG antibody level. There is sustained production of IgG agglutinins in subacute and chronic stage of brucellosis.[1]

A natural survey conducted in Saudi Arabia to asses the seroprevalence of brucellosis and the results revealed that out of 23,613 persons participated in the study , 3558 (15.0%) gave a positive reaction with STAT.[39]

While screening 292 blood donars in Chandigarh by slide agglutination method for *brucella* only one ample was positive and one was inconclusive. Of the above 292 samples 273 were screened for the presence of *brucella* antibodies by STAT. Only one sample was found to be positive at 1in160 dilution.[59]

Ofukwu *et al* [52] conducted a study among hospitalized patients in North central Nigeria, a total of 1040 samples were screened using the

RBPT and STAT. Of this 79 (7.6%) were seropositive by RBPT. The RBPT positive samples were further tested by STAT and 57 showed agglutination. The sensitivity of both these test are same.

The ELISA the advantage of identifying different classes of antibodies in comparison to other agglutination methods. This situation has an effect on the sensitivity, specificity and ultimately applicability of the method. This tests are relatively costlier tests in comparison to agglutination tests that requires equipment and experience.

The study conducted by Fatima et al in 2008 at Lahore, Pakistan[65] reported that the seroprevalence of Brucellosis among abattoir workers estimated using ELISA technique was 21.7%.

Kostoula *et al* [89] reported that when compared to the STAT, ELISA is most sensitive in the diagnosis of human brucellosis, because it detects IgM, IgA and IgG.

Ariza *et al*[29] reported that ELISA was a most sensitive and specific technique than SAT for the diagnosis of human brucellosis. Gazapo *et al* [90] stated that IgM and IgG ELISA can be used for epidemiological investigations.

Agasthaya *et al* [68] reported that out of 618 samples screened, 2.26%.2.26% and 15.69% were positive by STAT, RBPT and ELISA respectively. However, the overall prevalence was 15.69% by indirect ELISA. This ELISA has high sensitivity and specificity.

Hassan JS *et al* reported that ELISA was the most appropriate test when compared with STAT because ELISA detects more IgG positive cases than STAT.

Orduna *et al* [37] conducted a study and the results revealed that the sensitivity of Immuncapture agglutination test, Coombs test in STAT in the diagnosis of brucellosis was found to be 95.1 %, 91.5 % and 65.8 % respectively.

In a comparative study conducted by Prado *et al* [91] *Brucella* capt, STAT and Coombs anti-*Brucella* test were compared with Ig G, Ig A and Ig M ELISA tests.The sensitivity and specificity of Brucellacapt and Coombs anti-*brucella* were similar to one another. In the follow-up of the treatment, the antibody titers determined via these tests were close to ELISA and it was concluded that they were well correlated.

Mantur B *et al* [31] conducted a study in a 92 patients having presumptive diagnosis of brucellosis and the diagnosis was confirmed by blood cuture, STAT, 2-ME and ELISA. Blood culture was positive in 31 (33.6%), STAT in 23 (25%) cases, and 2-ME was positive in 21( 22.8%) whereas ELISA IgM and IgG together were found positive in 56 (60.9%) cases. The sensitivity and specificity for ELISA were found to be 100% and 71.31% respectively. A statistically significant difference was noticed in the performance of ELISA over traditional agglutination tests.

A comparative study conducted by Araj *et al* [35]argued that the ELISA method should be preferred because in chronic and complicated cases, STAT and Rose Bengal tests might miss a serious portion of positive cases

A comparative study of laboratory diagnostic tests of human brucellosis by Sathyanarayan MS *et al* [92] found that among the cases of PUO, none of them grew any isolate of *Brucella* in blood cultures. Seven were seropositive by agglutination tests and 13 of the 42 cases yielded positive results with ELISA .Serological tests are more sensitive as compared to blood culture. ELISA is the most sensitive test .

Vaso Taleski [93] conducted a study to analyse the various diagnostic methods of human brucellosis from patients at different stages of the disease showed the sensitivity of culture was 17.7%, RBPT 96%, STAT 84%, Coombs 86%, cELISA 98%, ELISA 98%, and - Specificity of: culture 100%, RBPT 97%, SAT 100%, Coombs 100%, cELISA 98%, ELISA 100%. The test results revealed that ELISA is the best serological test for diagnosis of human brucellosis.

# 4. MATERIALS AND METHODS

The present study was conducted at Tirunelveli Medical College and Hospital, Tirunelveli, Tamilnadu from September 2011 to August 2012 to detect the seroprevalence of brucellosis by measuring the *Brucella* IgG antibody by ELISA.

## 4.1 Materials

### 4.1.1 Study population

**a. Animal handlers.**

A total of 130 blood samples from veterinary surgeons, veterinary hospital workers and farmers of Tirunelveli district were collected.

**b. Control group**

A total of 130 blood samples were collected from Doctors, Post graduate students, lab technicians and clerical staffs of Tertiary care hospital of Tirunelveli.

Thus a total of 260 individuals participated in this study.

### 4.1.2 Ethical clearance

As this study involved collection of blood from human beings, ethical committee clearance was obtained before the commencement of the study.

### 4.1.3 Informed consent

Informed consent was obtained from all the persons who were involved in this study before blood collection.

### 4.1.4 Questionnaire

A questionnaire was used to obtain information from the study. (Annexure-I )

### 4.1.5 Sample collection and serum separation

About 3-5 ml of venous blood was collected from all the persons who involved in this study with aseptic precautions in sterile collection tubes and labelled properly. They were allowed to clot at room temperature for 30 minutes.

### 4.1.6 Storage of serum

Serum samples were stored at -20°C in deep freezer until testing. Repeated freezing and thawing was avoided.

### 4.1.7 Bio medical waste management

As the materials handled were highly infectious, proper biomedical waste management according to the regional guidelines was followed throughout the study.

## 4.2 ELISA kit

ELISA kit for *Brucella* IgG estimation was purchased from Vircell, Granada, Spain.

### 4.2.1 Principle

This ELISA method works on the principle that antibodies in the sample reacts with the antigen adsorbed on the polystyrene surface. Those unbound immunoglobulins are washed off. To this antigen – antibody complex, an enzyme-labelled anti-human globulin binds. Finally the substrate binds to the entire complex forming blue coloured soluble product which turns into yellow after adding the 0.5M sulphuric acid.

### 4.2.2 Contents of the kit

**Vircell *brucella* plate**

96 wells plate coated with LPS antigen of *B. abortus*,strain S-99.

**Serum diluent**

Phosphate buffer containing protein stabilizers and proclin.

**IgG positive control**

Positive control serum.

**IgG cut off control**

Cut off control serum

**IgG negative control**

Negative control serum .

**IgG conjugate**

Anti-human IgG peroxidase conjugate dilution in an orange-coloured proclin-containing buffer.

**TMB substrate solution**

Substrate solution containing TetraMethyl Benzidine (TMB).

**Stop reagent**

0.5 M $H_2SO_4$.

**Wash buffer**

20x washing solution, a phosphate buffer containing TweenR-20. and proclin.

### 4.2.3 Storage of kit

All the kit contents were stored at 2-8ºC.

## 4.3 Estimation of *Brucella* IgG Antibody by ELISA

### 4.3.1Requirements

- Kit contents
- Serum samples
- 5 µl and 100 µl micropipettes
- 100 µl 8 channel micropipette
- Micro tips, adhesive slips
- ELISA washer
- Thermostablised incubator or water bath
- Spectrophotometer with a 450 nm measuring filter and a 620 nm reference filter
- Distilled water
- Disposable gloves, absorbent papers, absorbent pad
- Disinfectant solution, black cover.

### 4.3.2 Preparation of the washing buffer

Distilled water was added to 50 ml of 20x washing solution and made upto 1 litre. The diluted wash buffer was stored at 2-8ºC.

### 4.3.3 Pre-requisites

- Incubator was set with 37±1ºC.
- All the reagents were brought to room temperature before use.
- The kit reagents were mixed thoroughly.

### 4.3.4 Procedure

- Plates were removed from the package.
- The samples were pre diluted in a separate tube, by adding 200 µl of serum diluent with 10 µl of sample. It was mixed homogenously with the pipette.
- First four wells were used for the controls - one for negative control, two for cut off serum and one for positive control. 100 µl of serum diluent was added to all the four wells and 5 µl of corresponding control sera were added.
- 105 µl of each diluted sample was dispensed into the appropriate wells. The plate was sealed and incubated at 37±1º C for 45 mints.
- The plate was washed with the diluted washing buffer for five times with the help of ELISA washer. Then the plate was blotted dry by tapping firmly onto absorbent paper.
- In each well, 100 µl of IgG conjugate solution was added**.**

- The ELISA plate was sealed and incubated at 37±1ºC for 30 mints.
- The seal was removed and the plate was washed with the diluted washing buffer for five times with the help of ELISA washer. Then the plate was blotted dry by tapping firmly onto absorbent paper.
- Then 100 µl of TMB substrate solution was added in all wells and incubated at RT for 20 mints and protected from light by keeping the plate in the dark environment.
- In each well, 50 µl of 0.5M $H_2SO_4$ was added and the it was read with a help of ELISA reader with spectrophotometer at 450/620 nm within 1 hour of stopping the reaction.

### 4.3.5 Validation protocol

| CONTROL | OD |
|---|---|
| Positive control | > 0.9 |
| Negative control | < 0.55 |
| Cut off control | < 0.7 x (O.D. Positive control) |
| | >1.5 x (O.D. Negative control) |

### 4.3.6 Interpretation of results

The mean O.D. for cut off serum was calculated.

**Antibody index = (sample O.D./ cut off serum mean O.D.) x 10**

| Index | Interpretation |
|---|---|
| **< 9** | **Negative** |
| **9-11** | **Equivocal** |
| **> 11** | **Positive** |

- Samples with equivocal results were repeated
- Samples with index < 9 were considered negative for IgG specific antibodies against *Brucella.*
- Samples with index > 11 were considered as positive for IgG specific antibodies against *Brucella.*

# Brucella IgG Elisa Kit


# Procedure

# Automatic micro plate washing instrument

# ELISA


# ELISA reader

# 5. RESULTS

## 5.1 Study population

### 5.1.1 Animal handlers

A total of 130 animal handlers were included in this study. They were veterinary surgeons and veterinary hospital workers who were working in and around Tirunelveli district and farmers who were residing in the same area. The study period was from September 2011 to August 2012.

### 5.1.2 Control group

A total of 130 non animal handlers were included in this study as controls. They were Doctors, Post graduate students, Laboratory technicians and Clerical staffs of Tertiary care hospital, Tirunelveli.

## 5.2 Statistical Analysis

The animal handlers were divided into two groups according to their *Brucella* IgG ELISA positive and negative results. They were matched according to their age and gender. The continuous variables between the two groups were compared by Student's unpaired 't' test and the categorical variables were compared by $\chi^2$ (Chi-square) test. The above statistical procedures were performed by the statistical package IBM SPSS statistics -20. The P values $<0.05$ determined the significance in two tailed tests.

**Table-1 Occupation wise distribution of animal handlers.**

| S.No | Occupation | No. of participants | Percentage |
|---|---|---|---|
| 1. | Veterinary surgeons | 80 | 61.5 |
| 2. | Veterinary hospital workers | 45 | 35.6 |
| 3. | Farmers | 5 | 3.9 |
| | Total | 130 | 100 |

The above table shows, among the 130 study population, 80 (61.5%) were veterinary surgeons, 45 (34.6%) were veterinary hospital workers and 5 (3.8%) were farmers.(Fig-1)

**Table-2 Occupation wise distribution of control group**

| S.No | Occupation | No. of participants | Percentage |
|---|---|---|---|
| 1. | Doctors | 34 | 26.1 |
| 2. | Post graduate students | 42 | 32.3 |
| 3. | Lab.Technicians | 21 | 16.1 |
| 4 | Clerical staffs | 33 | 25.4 |
| | Total | 130 | 100 |

Table -2 shows, out of 130 participants in the control group, 34(26.1%) were Doctors, 42(32.3%) were post graduate students , 21 (16.1%) were lab technicians and 33 (25.4%) were clerical staffs. (Fig-2)

**Fig. 1 Occupation wise distribution of animal handlers**

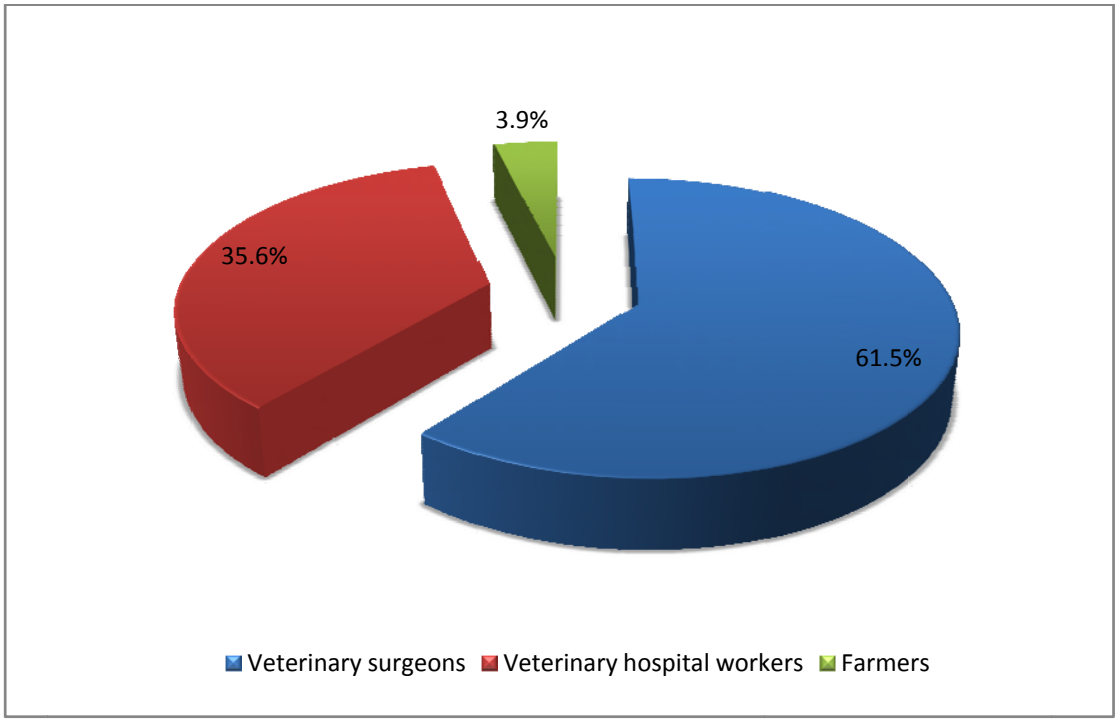



**Fig.2 Occupation wise distribution of control group**

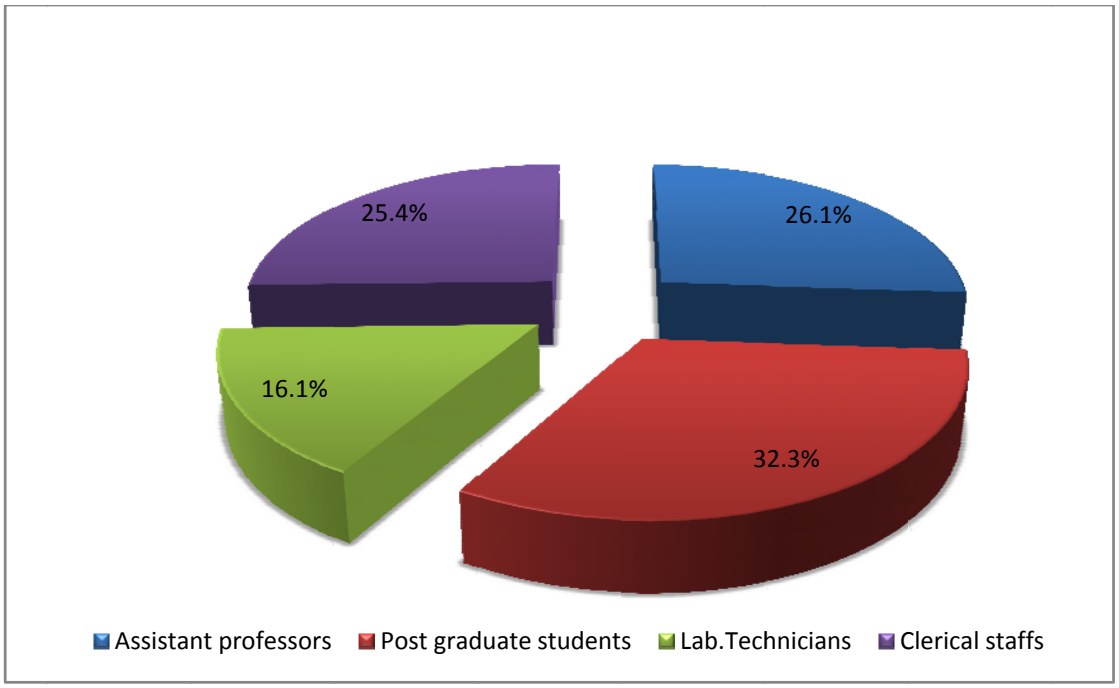


**Table- 3.Age and Sex wise distribution of animal handlers**

| .Age ( years) | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| 25-34 | 13 | 11.3 | 6 | 40 | 19 | 6.1 |
| 35-44 | 35 | 30.4 | 8 | 53.3 | 43 | 17.7 |
| 45-54 | 56 | 48.7 | 0 | 0 | 56 | 43.1 |
| 55-64 | 11 | 9.6 | 1 | 6.7 | 12 | 33.1 |
| Total | 115 | 100 | 15 | 100 | 130 | 100 |

Among the 130 participants,115 (88.5%) were males and 15 (11.5%) were females. Of this 13 (11.3%) males and 6 (40%) females were in the age group of 25-34 years, 35 (30.4%) males and 8 (53.3%) females were in 35-44 years, 56 (48.7%) males and none of the females were in the age group of 45- 54 and 11 (9.6%) males, one (6.7%) female was in the age group of 55- 64 years. The mean age of the animal handlers was 44.5±8.3 years More males were in the age group of 45-54 years. (Fig-3)

**Table-4 Age and Sex wise distribution of control group.**

| Age ( years) | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| 25-34 | 22 | 31.0 | 36 | 61.0 | 58 | 44.6 |
| 35-44 | 33 | 46.5 | 17 | 28.8 | 50 | 38.5 |
| 45-54 | 14 | 19.7 | 6 | 10.2 | 20 | 15.4 |
| 55-64 | 2 | 2.8 | 0 | 0 | 2 | 1.5 |
| Total | 71 (54.6%) | 100 | 59 (45.4%) | 100 | 130 | 100 |

Among the 130 participants,71(54.6%) were males and 59 (45.4%) were females. Of this 22 (31%) males and 36 (61%) females were in the age group of 25-34 years, 33 (46.5%) males and 17 (28.8%) females were in 35-44 years, 14 (19.7%) males and 6 (10.2%) females were in the age group of 45- 54 and 2 (2.8%) males and none of the females were in the age group of 55- 64 years. (Fig-4)

**Fig, 3. Age and Sex wise distribution of animal handlers**

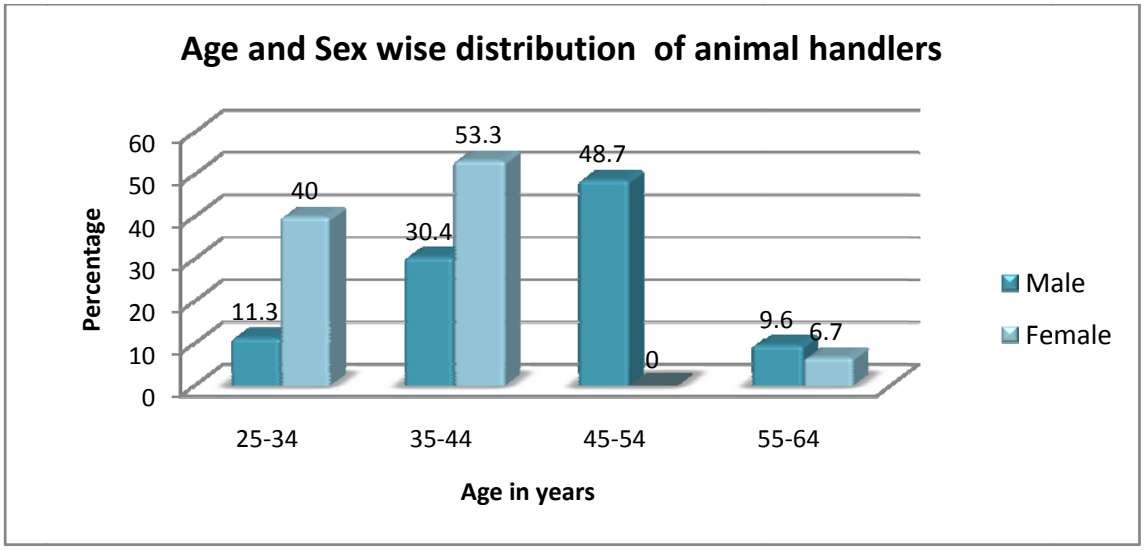



**Fig. 4 Age and Sex wise distribution of control group**

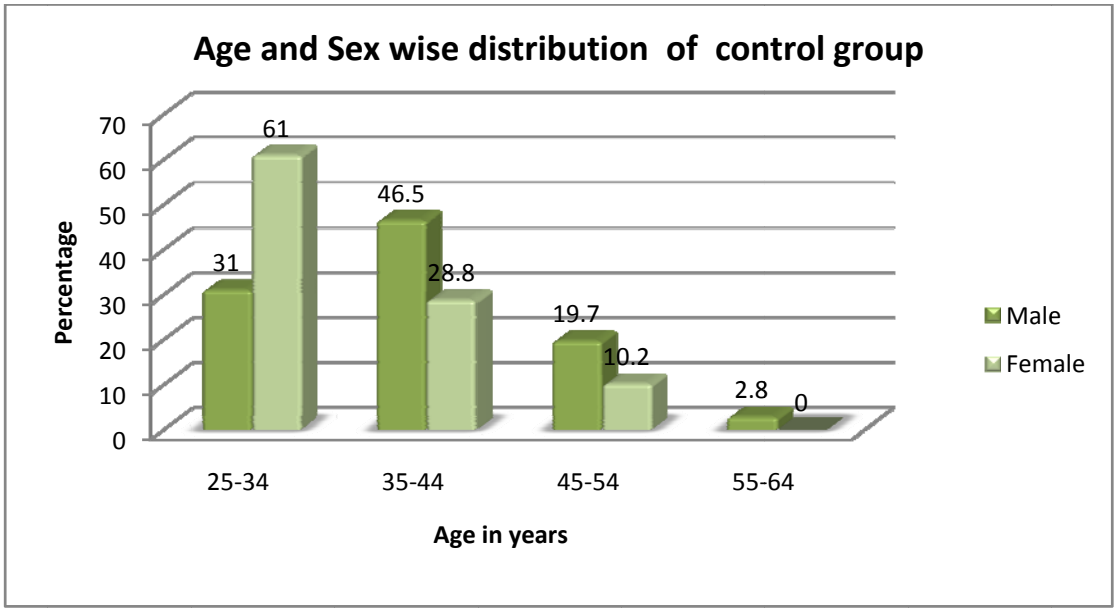


**Table-5 *Brucella* IgG positives and negatives among the animal handlers and control group.**

| ***Brucella* IgG ELISA Test** | **Animal handlers** | | **Control group** | |
|---|---|---|---|---|
| | **No** | **%** | **No** | **%** |
| Positive | 19 | 14.6 | 0 | 0 |
| Negative | 111 | 85.4 | 130 | 100 |
| Total | 130 | 100 | 130 | 100 |

The above table describes out of 130 study group, 19 (14.6%) were positive for *Brucella* IgG antibody and 111 (85.4%) were negative for IgG antibody. Among the 130 control group none were positive for *Brucella* IgG antibody. Seropositivity between animal handers and control group (non-animal handlers) was staistically significant ( $P < 0.0001$). (Fig-5)

**Table- 6. Gender wise distrbution of seropositives and seronegatives among animal handlers.**

| ***Brucella* IgG ELISA** | **Male** | | **Female** | |
|---|---|---|---|---|
| | **No** | **%** | **No** | **%** |
| Seropositive n =19 | 18 | 94.7 | 1 | 5.3 |
| Seronegative n=111 | 97 | 87.4 | 14 | 12.6 |

The above table shows, out of the 19 seropositive individuals 18 (94.7%) were males and one (5.3%) was female. Among the 111 seronegatives 97 (87.4%) were males and 14 (12.6% ) were females.

The results revealed that there was no significant association of seropositivity between males and females. (P>0.05) (Fig-6)

**Fig. 5 *Brucella* IgG positives and negatives among the animal handlers and control group**

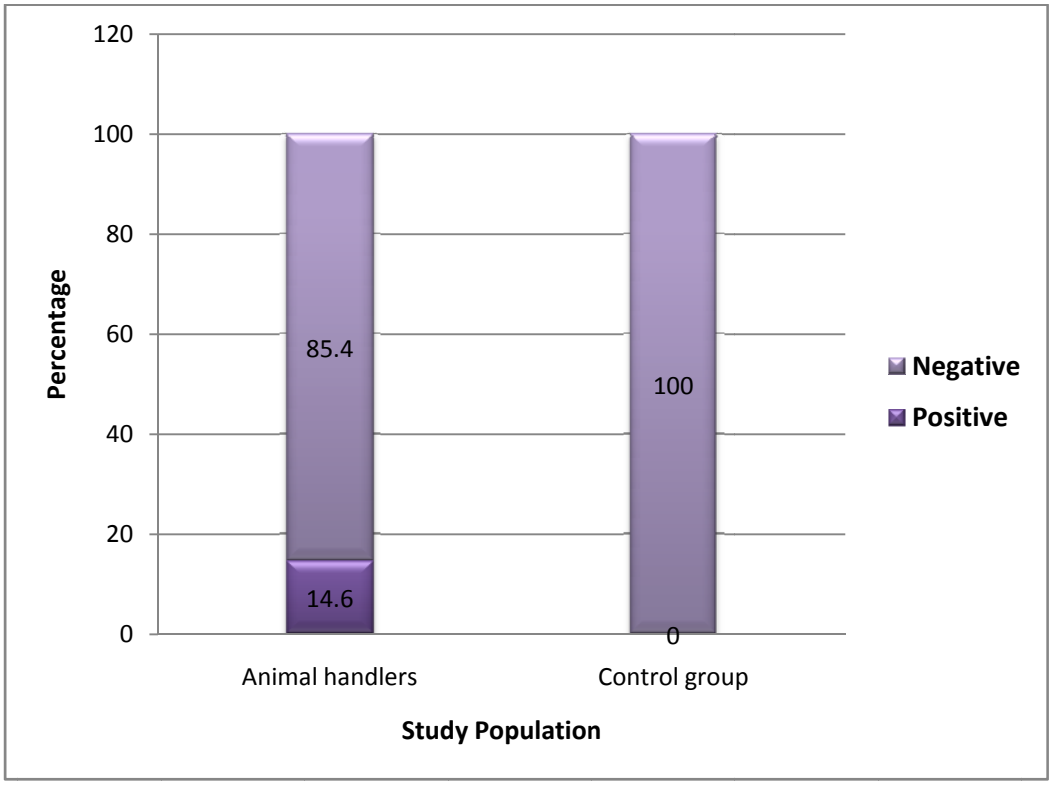



**Fig. 6 Gender wise distrbution among seropositives and seronegatives among animal handlers.**

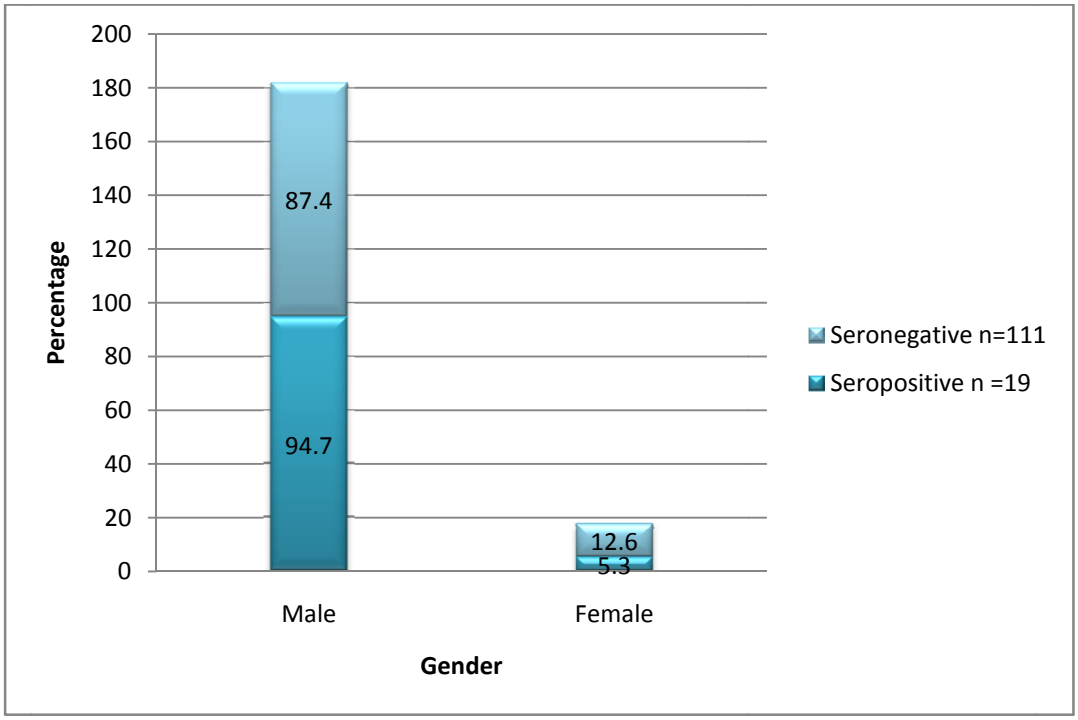


**Table - 7. Age wise distribution of seropositives and seronegatives among animal handlers.**

| Age (years) | Positive | | Negative | |
|---|---|---|---|---|
| | No | % | No | % |
| 25-34 | 0 | 0.0 | 19 | 17.1 |
| 35-44 | 3 | 15.8 | 40 | 36.0 |
| 45-54 | 11 | 57.9 | 45 | 40.6 |
| 55-64 | 5 | 26.3 | 7 | 6.3 |
| Total | 19 | 100.0 | 111 | 100.0 |

Table -7 shows that the maximum number seropositives 11(57.9%) were in the age group of 45-54 years. The mean age of the seropositive individual was 50.4 ± 4.6 and the same of the seronegative was 43.5 ±8.4 years. The difference between them was 6.9 years and the age was statistically significant (P<0.01).(Fig-7)

**Table-8. Occupation wise distribution of seropositives and seronegatives among animal handlers.**

| Occupation | Seropositive | | Seronegative | |
|---|---|---|---|---|
| | No | % | No | % |
| Veterinary Surgeon | 11 | 57.9 | 69 | 62.2 |
| Vet. Hosp. Workers | 8 | 42.1 | 37 | 33.3 |
| Farmers | 0 | 0.0 | 5 | 4.5 |
| Total | 19 | 100 | 111 | 100 |

The above table describes, among the 19 seropositives 11 (57.9%) were veterinary Surgeons and 8 (42.1%) were veterinary hospital workers. None of the farmers were positive for IgG agglutinins.

Among the 111( 85.3%) seronegatives 69 (62.2%) were veterinary surgeons, 37 (33.3%) were veterinary hospital workers and 5 (4.5%) were farmers. The test results revealed that there was no significant association of seropositivity between the veterinary surgeons and veterinary hospital workers. ($P>0.05$).(Fig-8)

**Fig.7 Age wise distribution of seropositives and seronegatives among animal handlers**

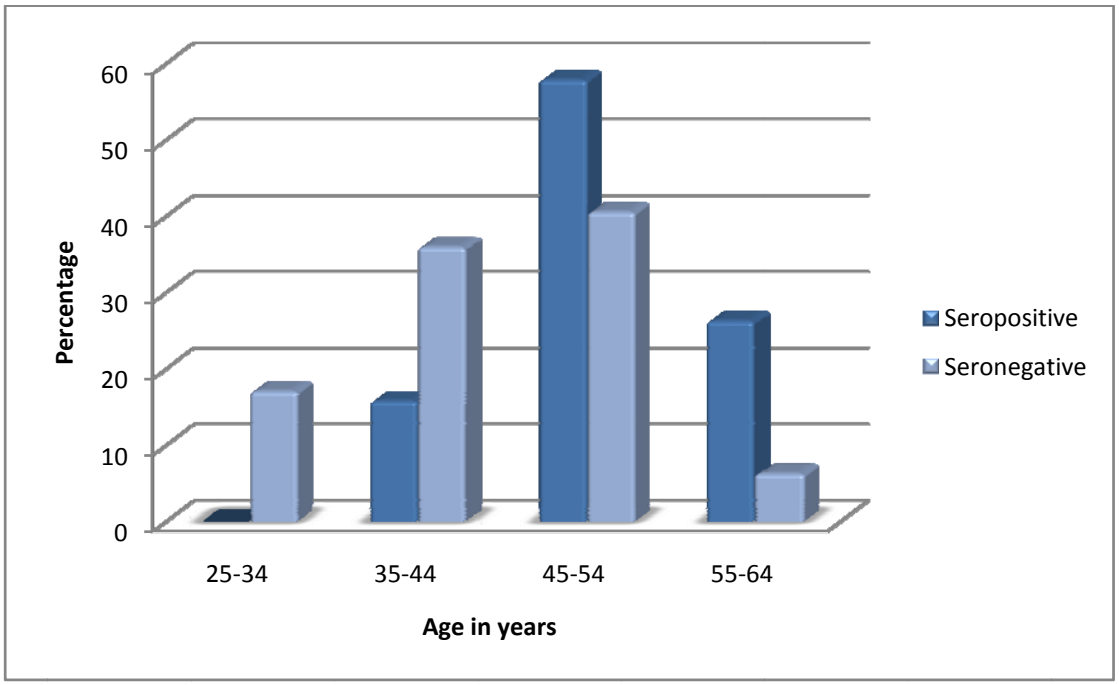



**Fig.8 Occupation wise distribution of seropositives and seronegatives among animal handlers**

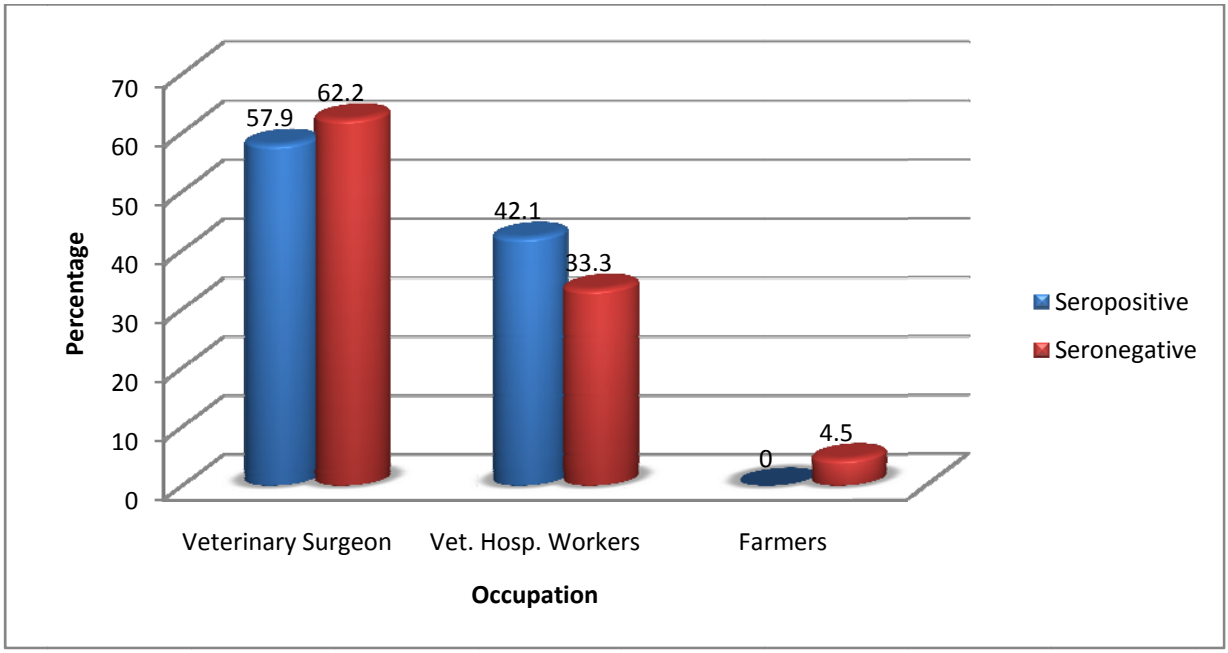


**Table - 9. Description and comparison of duration of exposure to animals**

| Duration of exposure (years) | Seropositive | | Seronegative | |
|---|---|---|---|---|
| | No | % | No | % |
| 5-14 | 1 | 5.3 | 40 | 36.0 |
| 15-24 | 8 | 42.1 | 45 | 40.6 |
| 25-34 | 10 | 52.6 | 21 | 18.9 |
| 35-44 | 0 | 0.0 | 5 | 4.5 |
| Total | 19 | 14.6 | 111 | 85.4 |

The duration of exposure to animals between the seropositives and seronegatives is shown in table-6.The more number of 10 (52.6%) seropositives had the duration of exposure of > 25 years. The mean duration of exposure in *brucella* seropositives was 25.3±5.0 years and the same in seronegative was 18.0 ± 7.9 years. The difference between them was statistically significant (P<0.001). (Fig-9)

**Table- 10. Handling of animal vaccine for *Brucella* among animal handlers.**

| Handling of *Brucella* vaccine | Positive | | Negative | | Total |
|---|---|---|---|---|---|
| | No | % | No | % | No |
| Yes (Handled) | 13 | 68.4 | 8 | 7.2 | 21 |
| No ( did Not handle) | 6 | 31.6 | 103 | 92.8 | 109 |
| Total | 19 | 100 | 111 | 100 | 130 |

Table-10 describes, among the130 animal handlers 21 had handled the *brucella* vaccine for animals and 109 had not handled the vaccine. Out of the 21 vaccine handled persons13 were seropositive and 8 were seronegative for *Brucella* IgG antibody.

Among the 19 seropositives, 13 (68.4%) had handled the vaccine and 6 (31.6%) had not handled the vaccine. Out of 111 seronegatives only 8 (7.2%) had handled the vaccine and 103 (92.8%) had not handled the vaccine. Thus the association between handling of vaccine and seropositivity is statistically significant ($P<0.0001$). (Fig-10)

**Fig. 9 Description and comparison of duration of exposure to animals**

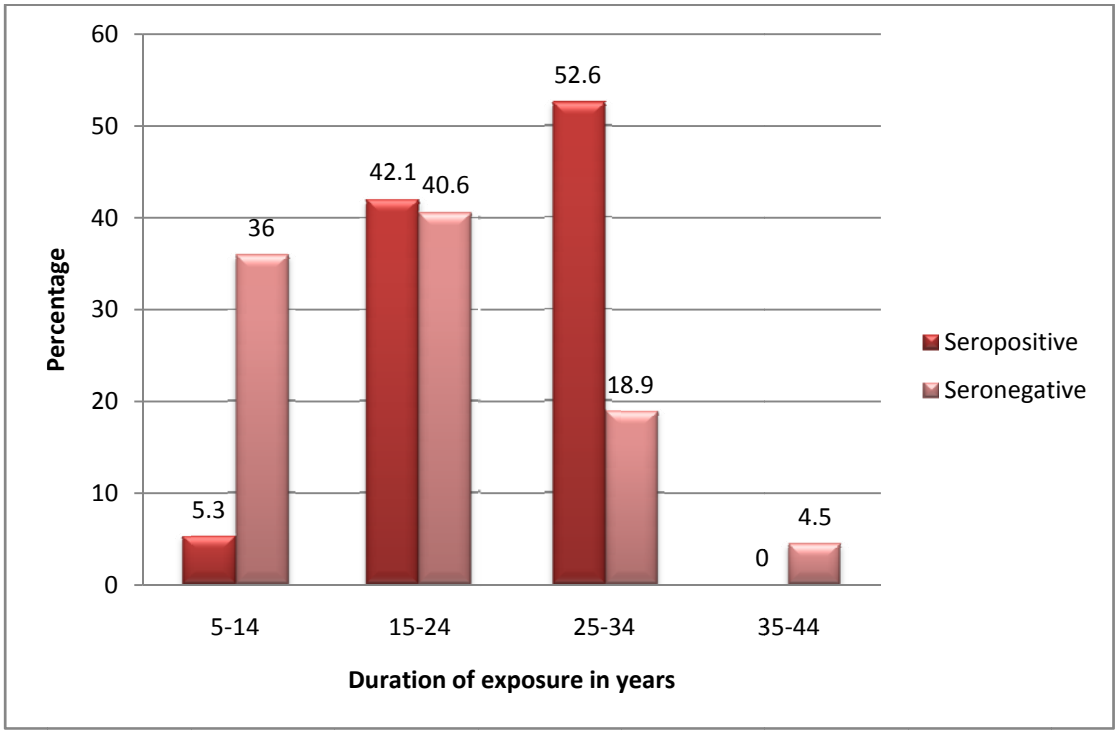



**Fig. 10 Handling of animal vaccine for *Brucella* among animal handlers**

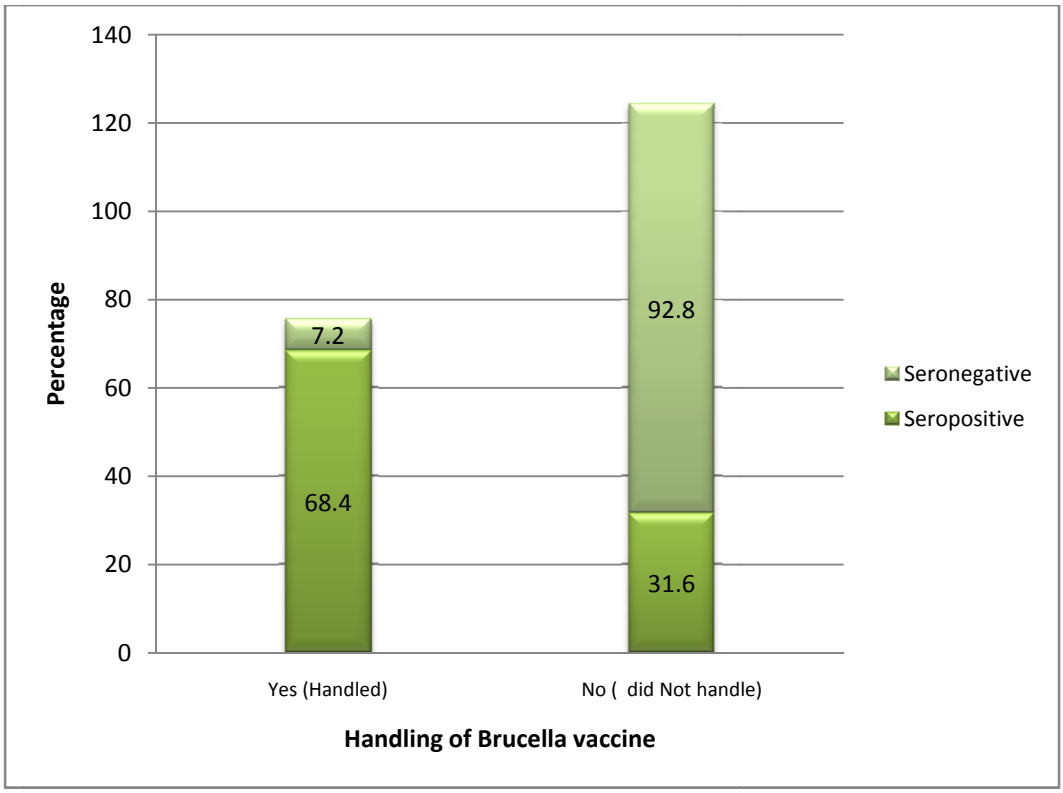


**Table- 11. Consumption of raw dairy products among animal handlers.**

| Raw dairy consumption | Positive | | Negative | | Total |
|---|---|---|---|---|---|
| | No | % | No | % | No |
| Yes | 11 | 57.9 | 25 | 22.5 | 36 |
| No | 8 | 42.1 | 86 | 77.5 | 94 |
| Total | 19 | 100 | 111 | 100 | 130 |

Among the total of 130 study group, 36 had history of consumption of raw dairy products and 94 had no such history.

Out of the total 19 *brucella* IgG positives, 11(57.9%) had consumed the raw dairy products and 8 (42.1%) had not consumed the products. Among the111 seronegative individuals, 25 (22.5%) had the history of consumption and 86 (77.5%) had not consumed the raw dairy products. The test results revealed that there was significant association between seropositivity and consumption of raw dairy products. (Fig-11)

**Table- 12. Awareness of brucellosis among animal handlers.**

| Awareness of brucellosis | Seropositive | | Seronegative | | Total |
|---|---|---|---|---|---|
| | No | % | No | % | No |
| Yes | 11 | 57.8% | 95 | 85.6 | 106 |
| No | 8 | 42.2 | 16 | 14.4 | 24 |
| Total | 19 | 100 | 111 | 100 | 130 |

Table -12 shows, among the 130 animal handlers 106 were aware of the disease and 24 were not aware of the disease. Among those who were aware of the disease 11 were seropositive and 95 were negative for *brucella* IgG antibody. Out of 24 persons who were not aware of the disease 8 were positive and 16 were negative.

Among the total 19 IgG positive individuals, 11 (57.8%) were aware of the disease and 8 (42.2%) were not aware of the disease. Out of the 111 seronegatives 95 (85.6%) were aware of brucellosis and 16 (14.4%) were not aware of it. There was significant ($P<0.001$) association between awareness of disease and decreased seropositivity.

**Fig. 11 Consumption of raw dairy products among animal handlers.**

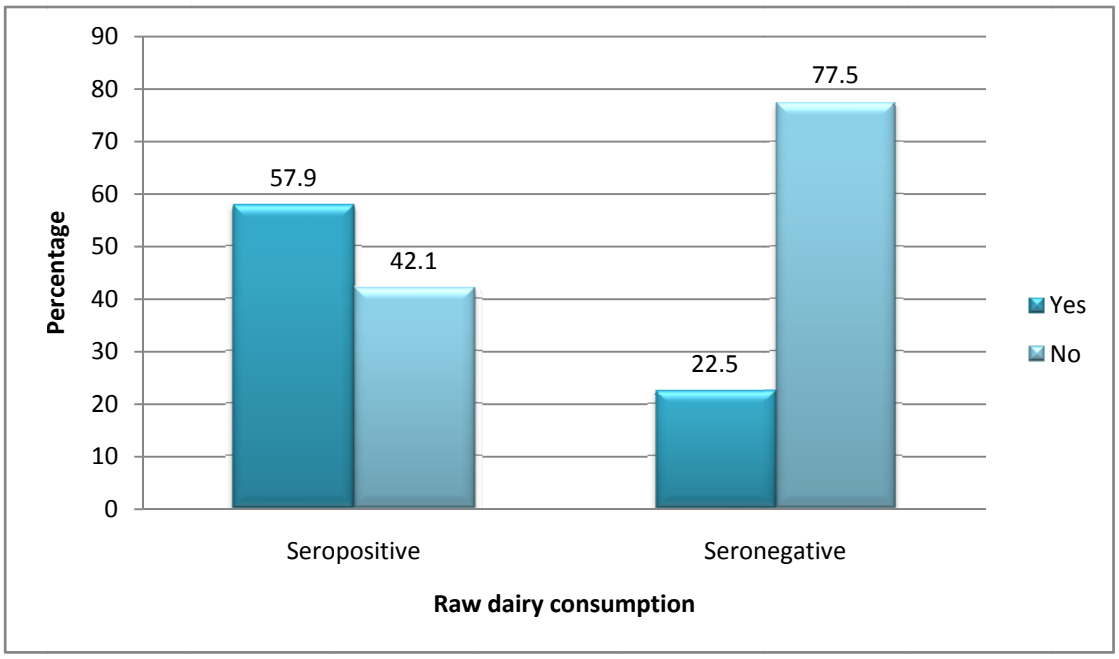



**Fig.12 Awareness of brucellosis among animal handlers**

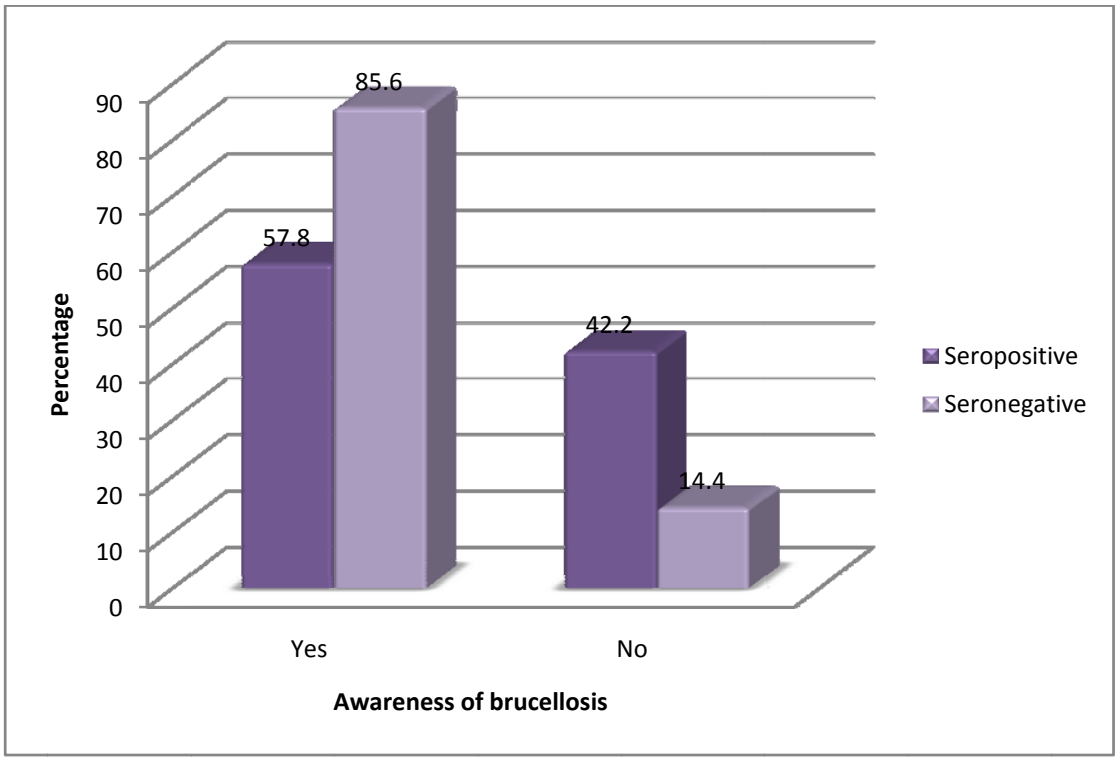
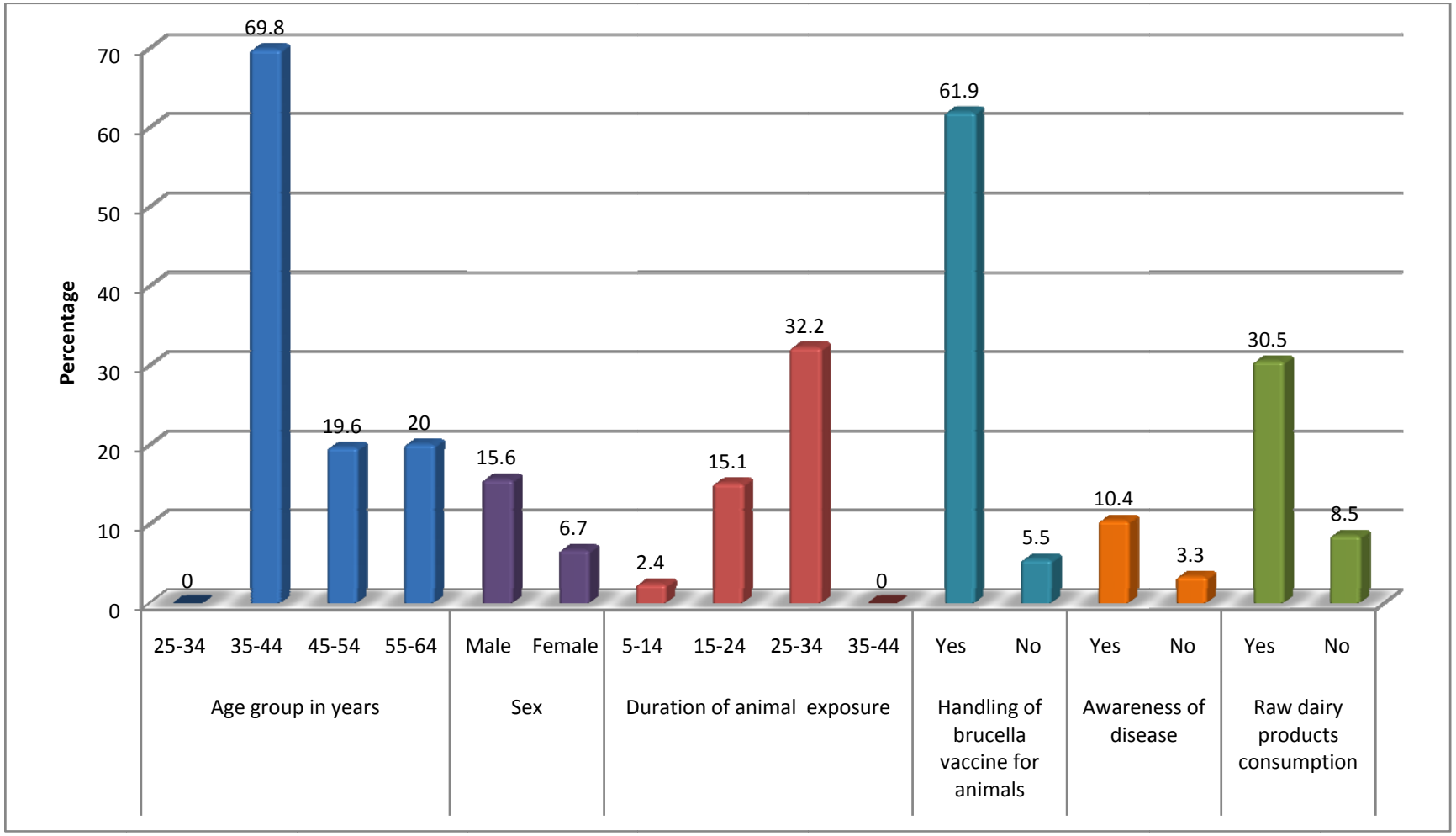


**Table-13. Socio demographic profile of seropositives.**

| S.No | Variable | Total | ELISA positive | | P value |
|---|---|---|---|---|---|
| | | | No | % | |
| 1. | Age group in years | | | | |
| | 25-34 | 19 | 0 | 0 | |
| | 35-44 | 43 | 3 | 69.8 | |
| | 45-54 | 56 | 11 | 19.6 | |
| | 55-64 | 1 | 5 | 20.0 | |
| 2. | Sex | | | | |
| | Male | 115 | 18 | 15.6 | P>0.05 |
| | Female | 15 | 1 | 6.7 | |
| 3. | Duration of animal exposure | | | | |
| | 5-14 | 41 | 1 | 2.4 | |
| | 15-24 | 53 | 8 | 15.1 | P<0.001 |
| | 25-34 | 31 | 10 | 32.2 | |
| | 35-44 | 5 | 0 | 0 | |
| 4. | Handling of brucella vaccine for animals | | | | |
| | Yes (Handled) | 21 | 13 | 61.9 | P<0.0001 |
| | No (Not handled) | 109 | 6 | 5.5 | |
| 5. | Awareness of disease | | | | |
| | Yes | 106 | 11 | 10.4 | P<0.001 |
| | No | 24 | 8 | 3.3 | |
| 6. | Raw dairy products consumption | | | | |
| | Yes | 36 | 11 | 30.5 | P<0.001 |
| | No | 94 | 8 | 8.5 | |

Table-13 summarises the important sociodemographic profile of the total 19 *Brucella* IgG positive animal handlers and their significance in association with the disease.

**Fig. 13 Socio demographic profile of seropositives**



# 6. DISCUSSION

## 6.1 Study population

130 animal handlers ( high risk group) and 130 controls were included in the present study to detect the seroprevalence of brucellosis.

In the high risk group, 80 ( 61.5%) veterinary surgeons, 45(35.6%) veterinary hospital workers and 5 (3.9%) farmers of Tirunelveli district , were tested for seroprevalence of brucellosis. It consisted of more males (88.5%) than females (11.5%) and many (56) participants were in the age group of 45-54 years.

In the control group, 34 (26.1%) Doctors, post graduate students, 21(16.1%) lab technicians and 33(25.4%) clerical staff were included. This group comprised of more males ( 54.6%) than females (45.4%). 58 participants were in the age group of 25 – 34 years and 50 were in the age group of 35-44 years .

## 6.2 Seroprevalence

The seroprevalence of brucellosis was found to be 14.6 % among these animal handlers which is suggestive increased exposure to *Brucella* species. In the control group the seroprevalence was found to be nil. This could be due to the fact that the control group had no occupational contact with animals and also because of an urban life style and safe hygienic measures like consumption of pasturised dairy products. On the other hand, the animal handlers may have developed

increase in titre of humoral antibodies as a result of repeated exposure to the agent.

The disease produced by *Brucella* results in protean clinical manifestation and low mortality. However, the morbidity of brucellosis is high because the disease process is long and incapacitating. In this study, all of the screened individuals were asymptomatic. The *Brucella* antibodies detected in asymptomatic individuals may be due to a history of repeated exposure to antigenic stimuli or inactive brucellosis.

The ELISA test which was used to detect IgG antibodies in the present study is very sensitive. This method can be used for the detection of immunoglobulin isotypes and gives better interpretation regarding the clinical situation and overcome the false negative and false positives which may arise in agglutination tests. More over this test is suited for screening large number of samples.

These antibodies seems to persist for a longer period or until they maintain contact with infected animals. This finding was similar to the studies among high risks group persons by Agasthya *et al* (15.69%) in Karnataka, [68] Bhat et al (5.8 to 14.3% ) in Belgum [63] and Alsekait (15%) in Saudi Arabia.[66] Reports from various developing countries in Mediterranean basin revealed that the seroprevalence ranged from 8% in Jordan [40] to 12 % in Lebanon and Kuwait,[41,42] 18% in Uganda and 13% [43] in Nigeria.

In comparison to other studies by Fatima Mukhtar (21.7%) in Pakistan,[65] Rana *et al* (27.7%), Kumar *et al* (28.57%) [84] and Cadmus S.I.B *et al* (31.82%)[48] in Ibadan, Southwestern Nigeria, the seroprevalence in the present study was low. This could be due to the difference in geographical location, variation in existence of disease among animals and personal hygienic practice and social habits.

But the seroprevalence was found to be high when compared to few Indian studies done by Thakur and Thalipal (4.97%) in Uttaranchal[80] and Ajay et al (1.14%) in Kerala.[61] This could be because of intense agricultural activities, cattle rearing, dairy farming and close contact with animals in the study area.

**6.3 Sex**

In the present study the seropositivity was found to be higher in males (94.7%) than females (5.3%). This is in concordance with the study done by A.S. Agasthya *et al* in Karnataka,[68] Jama ayah MZ *et al* in Malaysia[51] and Moti Yohannes *et al* in Ludhiana [57] where prevalence was more common among males than females. Whereas Kapoor *et al* [72] and Hussein *et al* [73] reported in their study that increased seropositivity in females when compared to males. However in the present study seropositivity between male and females was not statistically significant, which may be due to less number of females in the occupationally exposed study group.

## 6.4 Age

The seroprevalence of Brucellosis among animal handlers in the present study was highest in 45-54 (57.9%) years of age.Agasthya *et al* [68] reported highest prevalence of 45.36% among 41-50 years age group and Fatima *et al* [65] observed that the highest number of seropositives were in the age of 51 to 60 years . Ramos *et al*,[66] Jama'ayah MZ *et al* [51] reported that, animal handlers those who were in the age of 40 and above years had the highest prevalence. Whereas Kadri *et al* [55] reported that those were in the age of 21 to 30 years had increased (43%) seropositivity.

As brucellosis is an occupational disease, animal handlers > 40 years are at a greater risk of acquiring the disease due to prolonged duration of exposure to live stocks. The present study showed there was significant association between age group and seropositivity of disease.

## 6.5 Occupation

In the present study , 57.9% of veterinary surgeons and 42.1% of veterinary hospital workers were positive for *brucella* antibody. This is in concordance with the study done by Araj GF *et al* [41] who reported that veterinary surgeons had the highest prevalence (50%) among the occupationally exposed group. Agasthya *et al* [68] also observed that 59.8% of veterinary professionals and 37.1% of veterinary hospital workers had positive serology for *Brucella* antibody. The high prevalence rate among

this group may be due to the fact that brucellosis is an occupational hazard to the veterinary professionals. Even though they are aware of the disease, they acquire brucellosis by cuts in the skin or splashes into the conjunctiva while handling the animal products and during intravaginal or intrauterine manipulations in infected animals or by accidental inoculation of animal vaccines.

Cadmus S.I.B *et al* [48] reported that butchers (63.63%) had the highest seroprevalence among the high risk groups. A study among abattoir workers in which, blood collectors had the highest (99.77%) seropositivity followed by 68.00% in butchers.[84] Abattoire workers and meat handlers who are in direct contact with carcasses of infected animals and raw meat have more chances of getting infection.

Farmers usually reside in close proximity with their livestocks. They get infection by contact with infected animals and also by handling the infected placenta, its membranes and their discharges. During the burial of the products of conception their hands and forearms get contaminated. However in this study seropositivity was not detected among farmers. It may be due to the fact that only few farmers were included in this study and also they were not exposed to the infected animals and their products.

## 6.6 Duration of work

In the present study 52.6% of seropositive individuals had worked in this profession for more than 25 years and 47.4% of them had worked less than 25 years. Similar results were obtained by Abo-Shehada *etal*[69] where the seropositivity of brucellosis was significantly high among those people who had worked for more than 22 years of duration. Karimi *et al*[76] and Sohaila *et al* reported strong correlation between the seropositivity of brucellosis and duration of work.

This is because many veterinary professionals have a long duration of practice in this field of veterinary medicine which brings them in close contact with animals. There is a strong correlation between duration of work and seropositivity in the present study.

## 6.7 Handling of animal vaccine for *Brucella*

In the present study 68.4% of seropositives had participated in vaccination of animals. There is a strong association between the participation in vaccination of animals and seropositivity. This is similar to the study by Moti Yohannes A *et al*[57] and Ashford DA *et al* [88] who reported significantly increased seroprevalence of brucellosis among the veterinarians who participated in vaccination of animals than those who did not.

The animal vaccine is prepared from the attenuated strain *B. abortus* S19 and is most commonly used to prevent animal brucellosis.

The high rate of seroprevalence among vaccine handlers would have been due to accidentally acquiring the infection through conjunctival splashes, cuts in skin and mucous membrane and infectious aerosol. [88]

### 6.8 Consumption of raw dairy products

In the present study 52.9% of seropositive individuals had consumed the raw dairy products . This was similar to the findings by Baba *et al*, [78] Hasanjani Roushan *et al* and Al-Fadhli *et al* observed that consumption of unpasturised dairy products was significantly associated with high seropositivity.There was significant association between seropositivity and consumption of unpasturised dairy products.[1]

Raw milk may also transmit other diseases to humans like bovine tuberculosis, salmonellosis and campylobacteriosis. Thus pasteurization of milk is an integerated control measure for multiple pathogens.

### 6.9 Awareness of brucellosis

In the present study out of the the 130 animal handlers, 106 were aware of the disease and 24 were not aware of the disease. 85.6 % of seronegative individuals were aware of brucelloisis All the veterinary surgeons and some of veterinary hospital worker were aware of brucellosis. There was significant association between awareness of disease and low seropositivity. This low seropositivity in persons who were aware of the disease may be due to the practice of proper hand hygienic measures and using personal protective equipments while

handling livestocks and their products.[65] Moti Yohannes *et al* [57] reported significant association between awareness of brucellosis and positive serology among high risk groups.

*Brucella* is one of the agent likely to be used as a biological weapon due to the fact that transmission through a spray is possible. It is a highly contagious bacteria and less than 100 organisms would be sufficient to produce infection in humans.

There is no vaccine for prevention brucellosis in humans. Human brucellosis should be controlled by vaccination of animals. Animal owners should be educated about the importance of vaccination of animals. Mass vaccination of cattles along with other measures such as screening and quarantine of infected live stocks can efficiently control the brucellosis in livestocks, thereby eventually reducing the transmission of infection to human population. Awareness about the preventive measures like barrier protection methods, hand hygiene should be created among the high risk persons in order to reduce the transmission of infection from animals to humans.[1]

# 7. SUMMARY

The present study was aimed at assessing the seroprevalence of brucellosis in Tirunelveli district of Tamil nadu and to analyse the risk factors associated with this disease.

130 controls and 130 animal handlers were included in the study. Blood samples were collected and brucella IgG antibodies were tested by the method of ELISA and the results were analysed.

- More number of participants were males in both groups.
- Among animal handlers most of them were in the age group of 45-54 years.
- Seroprevalence in the control group was nil.
- The seroprevalence among animal handlers was 14.6%
- Gender was not significantly associated with seropositivity. It may be due to less number of female veterinary professionals.
- Maximum number of seropositive cases occured in the 45-54 age group. The mean age of the seropositive individual was 50.4 ± 4.6.
- All the animal handlers included in this study were at risk irrespective of their nature of work.
- Duration of exposure to animals and handling the animals was a significant risk factor. Those who had the exposure of more than 25 years were at great risk. The mean duration of exposure for seropositivity was 25.3±5.0.

- Handling the animal vaccine for brucella was a significant risk factor. *B.abortus RB 51* is a live vaccine and the handlers get infected by inadvertent inoculation, conjunctival splashes and through aerosols.
- Consumption of raw dairy products was significantly associated with the seropositivity of brucellosis.
- Awareness about brucellosis and its route of transmission had an impact on with seroprevalence. The prevalence was low among the persons who were aware of the disease.
- The lack of human vaccine and effective control measures make it necessary for the veterinary health personnel, animal husbandary workers and health care providers to take protective measures.

# 8. CONCLUSION

- The present study showed a significantly higher seroprevalence among animal handlers compared to the controlgroup.
- Occupationally exposed persons to animal were at greater risk of acquiring brucellosis.
- Higher age groups, long duration of exposure to animals, handling and participation in vaccination of animals and consumption of raw dairy products were significantly associated with the increased seroprevalence.
- Awareness of brucellosis was significantly associated with the decreased seroprevalence.
- Gender and nature of work among the animal handlers did not show any significant association.
- The results of this study emphasizes that contact with animals and ingestion of contaminated animal products are important methods of transmission of brucellosis.
- To prevent the occupation related brucellosis the veterinary health professionals, animal husbandary workers and laboratory personnel should wear PPE and follow adequate hand hygienic measures.
- There is no human vaccine to prevent brucellosis in man. Since the disease has been seen as a major occupational hazard to the animal handlers, the successful way for the control of disease in human

beings is by proper elimination of the infected animals and vaccinating the non infected animals.

- Reporting the existence of brucellosis to health authorities and veterinary professionals is essential and knowledge about the prevalence of the disease can be used to prioritize a disease control strategy and to alert the health authorities.

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**Annexure-I**

DATA SHEET FOR COLLECTION OF SOCIO-DEMOGRAPHIC PROFILE

FOR P.G DISSERTATION WORK ON

**"Seroprevalence of brucellosis among animal handlers and analysis of risk factors"**

**1. General information**

Name : Age: Sex: M / F

Address : Occupation:

Residential Background : Urban / Semi urban / Rural

Socio Economic Status : Upper/ Middle/ Low.

Educational Status : Illiterate/ Elementary/Higher secon /Graduate

H/o handling animals : Yes / No

H/o consumption of : Raw milk/raw dairy products

**2. Occupational History**

i. Occupation : Animal Keeper/ Veterinarian/Livestock Inspector Butchers/ Abattoir Worker/ Vet.Hospital Worker Meat Seller/ Milk handlers/ Others.

ii. Type of animal handling : Cattle/ Sheep/Pet animals/ others

iii. Duration of contact with animal :

iv. H/o handling animal product : Yes/No

iv. H/o handling animal blood : Yes/No

v. H/o handling animal vaccine for *Brucella* : Yes/No

vi Awareness of brucellosis : Yes/No

**3.Lab investigations**

i. Sample : Blood for serum

ii. Investigation : IgG ELISA for *Brucella*

**4. Report**

Name : Age/ Sex :

Address : Sample : Blood

Received On :

Reported On :

| Test | Method | Sample OD | Reference OD Value |
|---|---|---|---|
| Brucella IgG | Indirect ELISA (Vircel) | | < 9 = Negative<br>9-11 = Equivocal<br>>11 = Positive |

**Interpretation:**

**Signature**

# ANNEXURE II

# MASTER CHART

## SOCIO-DEMOGRAPHIC DATA AND TEST RESULTS

| S.No | Age | Sex | Occupation | Duration of exposure to animals | Handling brucella vaccine | Handling of animal products | Raw dairy consumption | Awareness | IgG ELISA | Result |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 45 | M | Vet.Surgeon | 22 | No | yes | No | Yes | **1.69** | Negative |
| 2 | 51 | M | Vet.Surgeon | 26 | No | yes | No | Yes | **0.738** | Negative |
| 3 | 46 | M | Vet.Surgeon | 25 | No | yes | No | Yes | **9.5** | Negative |
| 4 | 45 | M | Vet.Surgeon | 22 | Yes | yes | Yes | Yes | **22.6** | Positive |
| 5 | 44 | M | Vet.Surgeon | 20 | No | yes | No | Yes | **1.5** | Negative |
| 6 | 50 | M | Vet.Surgeon | 23 | No | yes | No | Yes | **2.707** | Negative |
| 7 | 48 | M | Vet.Surgeon | 24 | Yes | yes | No | Yes | **17.32** | Positive |
| 8 | 46 | M | Vet.Surgeon | 25 | No | yes | No | Yes | **0.707** | Negative |
| 9 | 46 | M | Vet.Surgeon | 23 | No | yes | No | Yes | **1.2** | Negative |
| 10 | 48 | M | Vet.Surgeon | 24 | No | yes | Yes | Yes | **18.8** | Positive |
| 11 | 42 | M | Vet.Surgeon | 20 | No | yes | No | Yes | **4.183** | Negative |
| 12 | 42 | M | Vet.Surgeon | 20 | No | yes | No | Yes | **0.81** | Negative |
| 13 | 43 | M | Vet.Surgeon | 20 | No | yes | No | Yes | **3** | Negative |
| 14 | 29 | M | Vet.Surgeon | 10 | No | yes | No | Yes | **1.661** | Negative |
| 15 | 45 | M | Vet.Surgeon | 23 | No | yes | No | Yes | **0.83** | Negative |
| 16 | 45 | M | Vet.Surgeon | 5 | No | yes | No | Yes | **0.738** | Negative |
| 17 | 51 | M | Vet.Surgeon | 27 | Yes | yes | No | Yes | **19.5** | Positive |
| 18 | 48 | M | Vet.Surgeon | 25 | No | yes | No | Yes | **0.692** | Negative |
| 19 | 50 | M | Vet.Surgeon | 23 | No | yes | No | Yes | **1.107** | Negative |
| 20 | 50 | M | Vet.Surgeon | 27 | No | yes | No | Yes | **23.4** | Positive |
| 21 | 52 | M | Vet.Surgeon | 29 | No | yes | No | Yes | **5.07** | Negative |
| 22 | 38 | F | Vet.Surgeon | 14 | No | yes | No | Yes | 0.923 | Negative |
| 23 | 37 | M | Vet.Surgeon | 15 | No | yes | No | Yes | 0.8 | Negative |
| 24 | 51 | M | Vet.Surgeon | 30 | Yes | yes | No | Yes | 29.33 | Positive |
| 25 | 27 | F | Vet.Surgeon | 9 | No | yes | No | Yes | 4.266 | Negative |
| 26 | 40 | F | Vet.Surgeon | 14 | No | yes | No | Yes | 0.8 | Negative |
| 27 | 45 | M | Vet.Surgeon | 23 | No | yes | No | Yes | 0.92 | Negative |
| 28 | 38 | F | Vet.Surgeon | 15 | No | yes | No | Yes | 1.733 | Negative |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 29 | 31 | M | Vet.Surgeon | 12 | No | yes | No | Yes | 1.066 | Negative |
| 30 | 42 | M | Vet.Surgeon | 19 | No | yes | No | Yes | 1.146 | Negative |
| 31 | 53 | M | Vet.Surgeon | 28 | No | yes | No | Yes | 5.74 | Negative |
| 32 | 51 | M | Vet.Surgeon | 30 | Yes | yes | Yes | Yes | 8.82 | Negative |
| 33 | 29 | M | Vet.Surgeon | 10 | No | yes | No | Yes | 0.81 | Negative |
| 34 | 48 | M | Vet.Surgeon | 23 | Yes | yes | Yes | Yes | 0.72 | Negative |
| 35 | 27 | F | Vet.Surgeon | 6 | No | yes | No | Yes | 1.1 | Negative |
| 36 | 32 | M | Vet.Surgeon | 15 | No | yes | Yes | Yes | 1.09 | Negative |
| 37 | 30 | M | Vet.Surgeon | 10 | No | yes | No | Yes | 0.92 | Negative |
| 38 | 39 | M | Vet.Surgeon | 15 | No | yes | No | Yes | 0.97 | Negative |
| 39 | 34 | F | Vet.Surgeon | 12 | No | yes | Yes | Yes | 0.58 | Negative |
| 40 | 53 | M | Vet.Surgeon | 30 | Yes | yes | No | Yes | 13.1 | Positive |
| 41 | 32 | M | Vet.Surgeon | 10 | No | yes | No | Yes | 2.21 | Negative |
| 42 | 43 | M | Vet.Surgeon | 20 | Yes | yes | Yes | Yes | 22.36 | Positive |
| 43 | 40 | M | Vet.Surgeon | 18 | Yes | yes | No | Yes | 2.86 | Negative |
| 44 | 42 | F | Vet.Surgeon | 22 | No | yes | No | Yes | 1.92 | Negative |
| 45 | 57 | M | Vet.Surgeon | 32 | Yes | yes | No | Yes | 28.36 | Positive |
| 46 | 38 | M | Vet.Surgeon | 19 | No | yes | No | Yes | 0.84 | Negative |
| 47 | 51 | M | Vet.Surgeon | 25 | Yes | yes | No | Yes | 16.09 | Positive |
| 48 | 34 | M | Vet.Surgeon | 10 | No | yes | No | Yes | 0.65 | Negative |
| 49 | 42 | F | Vet.Surgeon | 15 | No | yes | No | Yes | 8.5 | Negative |
| 50 | 26 | F | Vet.Surgeon | 6 | No | yes | Yes | Yes | 0.826 | Negative |
| 51 | 42 | M | Vet.Surgeon | 18 | Yes | yes | No | Yes | 0.9 | Negative |
| 52 | 38 | M | Vet.Surgeon | 14 | No | yes | No | Yes | 0.85 | Negative |
| 53 | 30 | M | Vet.Surgeon | 8 | No | yes | Yes | Yes | 1.1 | Negative |
| 54 | 34 | F | Vet.Surgeon | 14 | No | yes | No | Yes | 0.89 | Negative |
| 55 | 37 | F | Vet.Surgeon | 14 | No | yes | No | Yes | 0.96 | Negative |
| 56 | 30 | F | Vet.Surgeon | 5 | No | yes | Yes | Yes | 1.96 | Negative |
| 57 | 38 | M | Vet.Surgeon | 20 | No | yes | Yes | Yes | 0.826 | Negative |
| 58 | 42 | M | Vet.Surgeon | 8 | Yes | yes | No | Yes | 1.173 | Negative |
| 59 | 42 | M | Vet.Surgeon | 15 | No | yes | Yes | Yes | 2.4 | Negative |
| 60 | 40 | M | Vet.Surgeon | 20 | No | yes | No | Yes | 0.93 | Negative |
| 61 | 42 | M | Vet.Surgeon | 15 | Yes | yes | Yes | Yes | 3.04 | Negative |
| 62 | 41 | M | Vet.Surgeon | 18 | Yes | yes | No | Yes | 4.58 | Negative |
| 63 | 43 | M | Vet.Surgeon | 17 | No | yes | No | Yes | 1.573 | Negative |
| 64 | 37 | M | Vet.Surgeon | 14 | No | yes | Yes | Yes | 1.13 | Negative |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 65 | 51 | M | Vet.Surgeon | 26 | No | yes | No | Yes | 0.973 | Negative |
| 66 | 42 | M | Vet.Surgeon | 30 | No | yes | No | Yes | 5.06 | Negative |
| 67 | 44 | M | Vet.Surgeon | 12 | No | yes | Yes | Yes | 0.68 | Negative |
| 68 | 27 | M | Vet.Surgeon | 8 | No | yes | No | Yes | 0.82 | Negative |
| 69 | 47 | M | Vet.Surgeon | 23 | No | yes | No | Yes | 3.38 | Negative |
| 70 | 44 | M | Vet.Surgeon | 23 | Yes | yes | Yes | Yes | 21.81 | Positive |
| 71 | 44 | M | Vet.Surgeon | 22 | No | yes | No | Yes | 0.6 | Negative |
| 72 | 36 | M | Vet.Surgeon | 11 | No | yes | No | Yes | 2 | Negative |
| 73 | 45 | M | Vet.Surgeon | 22 | No | yes | No | Yes | 1.63 | Negative |
| 74 | 53 | M | Vet.Surgeon | 26 | No | yes | No | Yes | 2.87 | Negative |
| 75 | 47 | M | Vet.Surgeon | 23 | No | yes | No | Yes | 4.27 | Negative |
| 76 | 43 | M | Vet.Surgeon | 20 | No | yes | Yes | Yes | 2.17 | Negative |
| 77 | 32 | M | Vet.Surgeon | 13 | No | yes | No | Yes | 2.914 | Negative |
| 78 | 27 | M | Vet.Surgeon | 8 | No | yes | No | Yes | 0.6 | Negative |
| 79 | 27 | M | Vet.Surgeon | 8 | No | yes | No | Yes | 0.82 | Negative |
| 80 | 65 | M | Farmer | 10 | No | No | No | Yes | 0.6 | Negative |
| 81 | 33 | M | Farmer | 12 | No | No | Yes | No | 0.51 | Negative |
| 82 | 37 | M | Farmer | 5 | No | No | No | No | 0.8 | Negative |
| 83 | 47 | M | Farmer | 15 | No | No | No | No | 0.77 | Negative |
| 84 | 35 | M | Farmer | 20 | No | No | Yes | Yes | 1.72 | Negative |
| 85 | 42 | M | Vet.Surgeon | 17 | No | Yes | No | No | 1.13 | Negative |
| 86 | 52 | M | Vet.Hosp work | 27 | No | Yes | Yes | No | 11.7 | Positive |
| 87 | 58 | M | Vet.Hosp work | 30 | No | Yes | No | No | 0.75 | Negative |
| 88 | 45 | M | Vet.Hosp work | 15 | No | Yes | No | No | 3.6 | Negative |
| 89 | 43 | M | Vet.Hosp work | 18 | No | Yes | Yes | Yes | 2 | Negative |
| 90 | 54 | M | Vet.Hosp work | 24 | No | Yes | No | No | 4.9 | Negative |
| 91 | 54 | M | Vet.Hosp work | 35 | No | Yes | No | No | 3.7 | Negative |
| 92 | 47 | M | Vet.Hosp work | 19 | No | Yes | Yes | Yes | 3.6 | Negative |
| 93 | 54 | M | Vet.Hosp work | 14 | No | Yes | No | No | 0.6 | Negative |
| 94 | 56 | M | Vet.Hosp work | 30 | No | Yes | No | Yes | 0.51 | Negative |
| 95 | 43 | F | Vet.Hosp work | 20 | No | Yes | No | Yes | 0.75 | Negative |
| 96 | 55 | M | Vet.Hosp work | 35 | No | Yes | Yes | Yes | 1.07 | Negative |
| 97 | 54 | M | Vet.Hosp work | 26 | No | Yes | No | Yes | o.7 | Negative |
| 98 | 49 | M | Vet.Hosp work | 33 | Yes | Yes | No | Yes | 5.32 | Negative |
| 99 | 54 | M | Vet.Hosp work | 22 | No | Yes | Yes | Yes | 0.8 | Negative |
| 100 | 58 | M | Vet.Hosp work | 22 | No | Yes | No | No | 0.77 | Negative |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 101 | 57 | M | Vet.Hosp work | 14 | No | Yes | Yes | Yes | 0.7 | Negative |
| 102 | 50 | M | Vet.Hosp work | 35 | No | Yes | No | Yes | 0.9 | Negative |
| 103 | 56 | F | Vet.Hosp work | 30 | No | Yes | Yes | No | 22.5 | Positive |
| 104 | 41 | M | Vet.Hosp work | 11 | No | Yes | No | Yes | 2.2 | Negative |
| 105 | 53 | M | Vet.Hosp work | 15 | No | Yes | No | Yes | 1.4 | Negative |
| 106 | 45 | M | Vet.Hosp work | 5 | No | No | No | No | 0.8 | Negative |
| 107 | 52 | M | Vet.Hosp work | 27 | No | Yes | No | Yes | 0.9 | Negative |
| 108 | 56 | M | Vet.Hosp work | 20 | yes | Yes | Yes | No | 15.8 | Positive |
| 109 | 45 | M | Vet.Hosp work | 5 | No | Yes | No | No | 3.977 | Negative |
| 110 | 40 | M | Vet.Hosp work | 5 | No | Yes | No | Yes | 1.5 | Negative |
| 111 | 50 | M | Vet.Hosp work | 6 | No | Yes | Yes | No | 3.76 | Negative |
| 112 | 42 | M | Vet.Hosp work | 12 | No | Yes | No | Yes | 1.3 | Negative |
| 113 | 52 | M | Vet.Hosp work | 25 | No | Yes | No | Yes | 1.6 | Negative |
| 114 | 52 | M | Vet.Hosp work | 15 | No | Yes | Yes | Yes | 1 | Negative |
| 115 | 53 | M | Vet.Hosp work | 27 | No | Yes | No | Yes | 5.7 | Negative |
| 116 | 57 | M | Vet.Hosp work | 30 | No | Yes | Yes | No | 11.5 | Positive |
| 117 | 56 | M | Vet.Hosp work | 36 | No | Yes | Yes | Yes | 5.46 | Negative |
| 118 | 49 | M | Vet.Hosp work | 35 | No | Yes | No | No | 2.4 | Negative |
| 119 | 45 | M | Vet.Hosp work | 25 | No | Yes | No | Yes | 0.7 | Negative |
| 120 | 46 | M | Vet.Hosp work | 20 | Yes | Yes | Yes | No | 14 | Positive |
| 121 | 43 | F | Vet.Hosp work | 5 | No | No | No | Yes | 1.8 | Negative |
| 122 | 48 | M | Vet.Hosp work | 15 | No | Yes | Yes | No | 1.4 | Negative |
| 123 | 43 | M | Vet.Hosp work | 12 | No | Yes | No | Yes | 0.5 | Negative |
| 124 | 53 | M | Vet.Hosp work | 13 | No | Yes | No | No | 1.08 | Negative |
| 125 | 53 | M | Vet.Hosp work | 27 | No | Yes | No | Yes | 3.4 | Negative |
| 126 | 54 | M | Vet.Hosp work | 28 | No | Yes | Yes | Yes | 1.05 | Negative |
| 127 | 50 | M | Vet.Hosp work | 30 | No | Yes | Yes | No | 21.4 | Positive |
| 128 | 44 | M | Vet.Hosp work | 12 | Yes | Yes | Yes | No | 17 | Positive |
| 129 | 55 | M | Vet.Hosp work | 24 | Yes | Yes | No | No | 26.3 | Positive |
| 130 | 51 | M | Vet.Hosp work | 20 | No | Yes | No | Yes | 6.11 | Negative |

**Abbreviations** M - Male, F - Female Vet.Hosp work - Veterinary Hospital worker
Vet.Surgeon - Veterinary Surgeon

# ANNEXURE II

# MASTER CHART

## SOCIO-DEMOGRAPHIC DATA AND TEST RESULTS

| S.No | Age | Sex | Occupation | Handling of animals | Duration of exposure to animals | Handling brucella vaccine | Raw dairy | Awareness | IgG ELISA | Result |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 35 | M | Doctors | No | Nil | Nil | No | Yes | **1.69** | Negative |
| 2 | 37 | M | Doctors | No | Nil | Nil | No | Yes | **0.738** | Negative |
| 3 | 39 | M | Doctors | No | Nil | Nil | No | Yes | **1.32** | Negative |
| 4 | 41 | M | Doctors | No | Nil | Nil | No | Yes | **0.8** | Negative |
| 5 | 36 | F | Doctors | No | Nil | Nil | No | Yes | **1.5** | Negative |
| 6 | 37 | M | Doctors | No | Nil | Nil | No | Yes | **2.707** | Negative |
| 7 | 36 | M | Doctors | No | Nil | Nil | Yes | Yes | **1.5** | Negative |
| 8 | 35 | M | Doctors | No | Nil | Nil | No | Yes | **0.707** | Negative |
| 9 | 35 | F | Doctors | No | Nil | Nil | No | Yes | **3.6** | Negative |
| 10 | 38 | M | Doctors | No | Nil | Nil | No | Yes | **1.2** | Negative |
| 11 | 39 | M | Doctors | No | Nil | Nil | No | Yes | **4.183** | Negative |
| 12 | 41 | M | Doctors | No | Nil | Nil | No | Yes | **0.81** | Negative |
| 13 | 40 | M | Doctors | No | Nil | Nil | No | Yes | **2.5** | Negative |
| 14 | 35 | F | Doctors | No | Nil | Nil | No | Yes | **1.78** | Negative |
| 15 | 36 | M | Doctors | No | Nil | Nil | Yes | Yes | **0.752** | Negative |
| 16 | 38 | M | Doctors | No | Nil | Nil | No | Yes | **0.869** | Negative |
| 17 | 38 | M | Doctors | No | Nil | Nil | No | Yes | **0.56** | Negative |
| 18 | 38 | F | Doctors | No | Nil | Nil | No | Yes | **0.34** | Negative |
| 19 | 53 | M | Doctors | No | Nil | Nil | No | Yes | **1.27** | Negative |
| 20 | 47 | M | Doctors | No | Nil | Nil | No | Yes | **4.23** | Negative |
| 21 | 52 | M | Doctors | No | Nil | Nil | No | Yes | **4.96** | Negative |
| 22 | 37 | F | Doctors | No | Nil | Nil | No | Yes | 0.759 | Negative |
| 23 | 37 | M | Doctors | No | Nil | Nil | No | Yes | 0.82 | Negative |
| 24 | 56 | M | Doctors | No | Nil | Nil | Yes | Yes | 3.56 | Negative |
| 25 | 36 | F | Doctors | No | Nil | Nil | No | Yes | 4.12 | Negative |
| 26 | 39 | F | Doctors | No | Nil | Nil | No | Yes | 0.8 | Negative |
| 27 | 42 | M | Doctors | No | Nil | Nil | No | Yes | 0.96 | Negative |
| 28 | 49 | F | Doctors | No | Nil | Nil | No | Yes | 1.23 | Negative |
| 29 | 45 | M | Doctors | No | Nil | Nil | No | Yes | 1.012 | Negative |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 46 | F | Doctors | No | Nil | Nil | No | Yes | 1.06 | Negative |
| 31 | 44 | M | Doctors | No | Nil | Nil | No | Yes | 4.01 | Negative |
| 32 | 48 | M | Doctors | No | Nil | Nil | No | Yes | 3.65 | Negative |
| 33 | 45 | F | Doctors | No | Nil | Nil | No | Yes | 0.81 | Negative |
| 34 | 49 | M | Doctors | No | Nil | Nil | No | Yes | 0.75 | Negative |
| 35 | 34 | F | Students | No | Nil | Nil | No | Yes | 1.023 | Negative |
| 36 | 26 | F | Students | No | Nil | Nil | Yes | Yes | 1.09 | Negative |
| 37 | 32 | F | Students | No | Nil | Nil | No | Yes | 0.756 | Negative |
| 38 | 27 | F | Students | No | Nil | Nil | No | Yes | 0.845 | Negative |
| 39 | 31 | F | Students | No | Nil | Nil | No | Yes | 0.562 | Negative |
| 40 | 36 | M | Students | No | Nil | Nil | No | Yes | 4.36 | Negative |
| 41 | 30 | F | Students | No | Nil | Nil | No | Yes | 2.65 | Negative |
| 42 | 25 | F | Students | No | Nil | Nil | No | Yes | 1.95 | Negative |
| 43 | 37 | F | Students | No | Nil | Nil | No | Yes | 1.65 | Negative |
| 44 | 40 | F | Students | No | Nil | Nil | No | Yes | 1.32 | Negative |
| 45 | 37 | M | Students | No | Nil | Nil | No | Yes | 1.96 | Negative |
| 46 | 27 | F | Students | No | Nil | Nil | No | Yes | 0.84 | Negative |
| 47 | 35 | F | Students | No | Nil | Nil | No | Yes | 2.15 | Negative |
| 48 | 38 | F | Students | No | Nil | Nil | No | Yes | 0.692 | Negative |
| 49 | 42 | F | Students | No | Nil | Nil | No | Yes | 0.98 | Negative |
| 50 | 29 | F | Students | No | Nil | Nil | No | Yes | 0.826 | Negative |
| 51 | 33 | M | Students | No | Nil | Nil | No | Yes | 1.2 | Negative |
| 52 | 32 | F | Students | No | Nil | Nil | No | Yes | 1.65 | Negative |
| 53 | 28 | F | Students | No | Nil | Nil | No | Yes | 1.2 | Negative |
| 54 | 33 | M | Students | No | Nil | Nil | No | Yes | 0.96 | Negative |
| 55 | 26 | M | Students | No | Nil | Nil | No | Yes | 0.925 | Negative |
| 56 | 25 | F | Students | No | Nil | Nil | No | Yes | 1.45 | Negative |
| 57 | 28 | F | Students | No | Nil | Nil | Yes | Yes | 0.85 | Negative |
| 58 | 32 | F | Students | No | Nil | Nil | No | Yes | 1.026 | Negative |
| 59 | 28 | M | Students | No | Nil | Nil | No | Yes | 1.89 | Negative |
| 60 | 28 | F | Students | No | Nil | Nil | No | Yes | 0.963 | Negative |
| 61 | 31 | F | Students | No | Nil | Nil | No | Yes | 3.04 | Negative |
| 62 | 29 | F | Students | No | Nil | Nil | No | Yes | 3.65 | Negative |
| 63 | 29 | M | Students | No | Nil | Nil | No | Yes | 1.256 | Negative |
| 64 | 28 | F | Students | No | Nil | Nil | No | Yes | 1.09 | Negative |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 65 | 29 | F | Students | No | Nil | Nil | No | Yes | 2.61 | Negative |
| 66 | 30 | M | Students | No | Nil | Nil | No | Yes | 3.65 | Negative |
| 67 | 26 | F | Students | No | Nil | Nil | No | Yes | 0.965 | Negative |
| 68 | 28 | F | Students | No | Nil | Nil | No | Yes | 1.86 | Negative |
| 69 | 31 | F | Students | No | Nil | Nil | No | Yes | 1.396 | Negative |
| 70 | 32 | M | Students | No | Nil | Nil | Yes | Yes | 2.95 | Negative |
| 71 | 34 | F | Students | No | Nil | Nil | No | Yes | 3.2 | Negative |
| 72 | 27 | F | Students | No | Nil | Nil | No | Yes | 1.956 | Negative |
| 73 | 28 | F | Students | No | Nil | Nil | No | Yes | 1.65 | Negative |
| 74 | 27 | F | Students | No | Nil | Nil | No | Yes | 2.65 | Negative |
| 75 | 31 | M | Students | No | Nil | Nil | No | Yes | 3.86 | Negative |
| 76 | 26 | F | Students | No | Nil | Nil | No | Yes | 2.56 | Negative |
| 77 | 39 | M | Lab tehnicians | No | Nil | Nil | No | Yes | 2.69 | Negative |
| 78 | 35 | M | Lab tehnicians | No | Nil | Nil | No | Yes | 0.768 | Negative |
| 79 | 42 | M | Lab tehnicians | No | Nil | Nil | No | Yes | 0.82 | Negative |
| 80 | 27 | M | Lab tehnicians | No | Nil | Nil | No | Yes | 0.6 | Negative |
| 81 | 31 | M | Lab tehnicians | No | Nil | Nil | No | No | 0.59 | Negative |
| 82 | 32 | F | Lab tehnicians | No | Nil | Nil | No | No | 0.81 | Negative |
| 83 | 29 | M | Lab tehnicians | No | Nil | Nil | No | No | 0.82 | Negative |
| 84 | 31 | M | Lab tehnicians | No | Nil | Nil | No | No | 1.68 | Negative |
| 85 | 29 | F | Lab tehnicians | No | Nil | Nil | No | No | 1.69 | Negative |
| 86 | 32 | M | Lab tehnicians | No | Nil | Nil | No | No | 1.25 | Negative |
| 87 | 31 | F | Lab tehnicians | No | Nil | Nil | No | No | 0.862 | Negative |
| 88 | 29 | M | Lab tehnicians | No | Nil | Nil | No | No | 3.56 | Negative |
| 89 | 28 | M | Lab tehnicians | No | Nil | Nil | Yes | Yes | 2 | Negative |
| 90 | 42 | F | Lab tehnicians | No | Nil | Nil | No | No | 1.32 | Negative |
| 91 | 37 | M | Lab tehnicians | No | Nil | Nil | No | No | 3.36 | Negative |
| 92 | 38 | F | Lab tehnicians | No | Nil | Nil | Yes | No | 2.5 | Negative |
| 93 | 35 | M | Lab tehnicians | No | Nil | Nil | No | No | 1.01 | Negative |
| 94 | 41 | M | Lab tehnicians | No | Nil | Nil | No | No | 0.86 | Negative |
| 95 | 29 | F | Lab tehnicians | No | Nil | Nil | No | No | 0.12 | Negative |
| 96 | 38 | M | Lab tehnicians | No | Nil | Nil | No | No | 2.01 | Negative |
| 97 | 37 | M | Lab tehnicians | No | Nil | Nil | No | No | 1.07 | Negative |
| 98 | 50 | F | Clerical staffs | No | Nil | Nil | No | No | 4.32 | Negative |
| 99 | 46 | M | Clerical staffs | No | Nil | Nil | No | No | 0.8 | Negative |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 100 | 37 | M | Clerical staffs | No | Nil | Nil | No | No | 0.96 | Negative |
| 101 | 29 | M | Clerical staffs | No | Nil | Nil | No | No | 0.845 | Negative |
| 102 | 48 | M | Clerical staffs | No | Nil | Nil | No | No | 0.925 | Negative |
| 103 | 46 | F | Clerical staffs | No | Nil | Nil | No | No | 4.2 | Negative |
| 104 | 25 | M | Clerical staffs | No | Nil | Nil | No | No | 2.65 | Negative |
| 105 | 27 | M | Clerical staffs | No | Nil | Nil | No | No | 1.98 | Negative |
| 106 | 47 | F | Clerical staffs | No | Nil | Nil | No | No | 2.65 | Negative |
| 107 | 39 | M | Clerical staffs | No | Nil | Nil | No | No | 3.6 | Negative |
| 108 | 38 | M | Clerical staffs | No | Nil | Nil | No | No | 1.65 | Negative |
| 109 | 42 | M | Clerical staffs | No | Nil | Nil | No | No | 3.654 | Negative |
| 110 | 32 | F | Clerical staffs | No | Nil | Nil | No | Yes | 1.12 | Negative |
| 111 | 28 | M | Clerical staffs | No | Nil | Nil | No | No | 3.48 | Negative |
| 112 | 26 | M | Clerical staffs | No | Nil | Nil | No | No | 1.65 | Negative |
| 113 | 33 | F | Clerical staffs | No | Nil | Nil | No | No | 1.6 | Negative |
| 114 | 34 | M | Clerical staffs | No | Nil | Nil | Yes | No | 1 | Negative |
| 115 | 34 | F | Clerical staffs | No | Nil | Nil | No | No | 2.5 | Negative |
| 116 | 34 | F | Clerical staffs | No | Nil | Nil | No | No | 2.65 | Negative |
| 117 | 32 | M | Clerical staffs | No | Nil | Nil | No | No | 2.45 | Negative |
| 118 | 35 | M | Clerical staffs | No | Nil | Nil | No | No | 3.254 | Negative |
| 119 | 31 | F | Clerical staffs | No | Nil | Nil | No | No | 0.845 | Negative |
| 120 | 37 | M | Clerical staffs | No | Nil | Nil | No | No | 4.1 | Negative |
| 121 | 37 | F | Clerical staffs | No | Nil | Nil | No | No | 2.96 | Negative |
| 122 | 45 | M | Clerical staffs | No | Nil | Nil | No | No | 1.52 | Negative |
| 123 | 48 | M | Clerical staffs | No | Nil | Nil | No | Yes | 0.658 | Negative |
| 124 | 39 | F | Clerical staffs | No | Nil | Nil | No | No | 1.64 | Negative |
| 125 | 47 | M | Clerical staffs | No | Nil | Nil | No | No | 3.584 | Negative |
| 126 | 56 | M | Clerical staffs | No | Nil | Nil | No | No | 2.54 | Negative |
| 127 | 45 | M | Clerical staffs | No | Nil | Nil | Yes | No | 1.365 | Negative |
| 128 | 49 | M | Clerical staffs | No | Nil | Nil | No | No | 1.346 | Negative |
| 129 | 37 | F | Clerical staffs | No | Nil | Nil | No | No | 1.355 | Negative |
| 130 | 50 | M | Clerical staffs | No | Nil | Nil | No | No | 3.265 | Negative |

**Abbreviations** M - Male, F - Female